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Disseminated *Conidiobolus incongruus* in a dog: A case report and literature review

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ABSTRACT

Conidiobolomycosis is a rare fungal disease of both humans and animals, occurring mainly in tropical and subtropical climates. We describe a disseminated fungal infection in a young, apparently immunocompetent dog who initially presented for antibiotic resistant pneumonia. Histopathology and mycology identified a *Conidiobolus* sp., further confirmed as *Conidiobolus incongruus* through DNA sequencing of D1/D2 regions. This is the first report of this species causing disease in dogs and the fifth reported infection in animals.

1. Introduction

Fungal infections due to *Conidiobolus* spp. are rare in dogs and cats; however, it has been more frequently reported in humans and livestock [1,2]. Conidiobolomycosis is characterized as a chronic, granulomatous fungal disease of the nasopharynx [1]. On rare occasion disease can spread to other areas of the head and neck as well as disseminate throughout the body [5–7]. Geographically the disease is distributed worldwide in tropical and subtropical climates [1]. Affected individuals are usually immunocompetent with locally extensive disease, as dissemination is rare [1–4]. The majority of infections in both humans and animals are caused by *Conidiobolus coronatus* with only a few due to *Conidiobolus lamprauges* or *Conidiobolus incongruus.* In humans, the handful of reported *C. incongruus* cases has predominantly involved the lungs or mediastinum, but pericardial, periorbital, orbitofacial and orofacial involvement have also been seen [8]. Only four reports are available in the literature describing infection with *C. incongruus* in animals and all four cases involve either deer or sheep [3–6]. Two of these are single case reports in deer [3,6], while the other two are herd outbreaks in sheep [4,5]. Here we report the first case of *C. incongruus* in a dog and review the literature regarding conidiobolomycosis in animals.

2. Case report

In September 2014, a 17-month-old intact male English Mastiff dog was presented to the Oklahoma State University Boren Veterinary Medical Teaching Hospital (OSU-BVMTH) for a one-month history of a productive cough, dyspnea, weight loss, fever, and lethargy. Patient was housed outdoors with access to a barn on acreage, including a pond, with cattle, horses, and cats. The patient had no travel history outside the state of Oklahoma. Respiratory signs (day 0) were noted 7 days prior to presentation at the primary care veterinarian's (pDVM) office.

Physical examination abnormalities on presentation to pDVM included fever 103.8 °F (38.89 °C) and tachypnea (56 breaths per min). A deep non-productive cough was appreciated along with increased effort on inspiration and congestion reported on auscultation. SNAP® 4Dx® serology (Idexx Laboratories, Westbrook,

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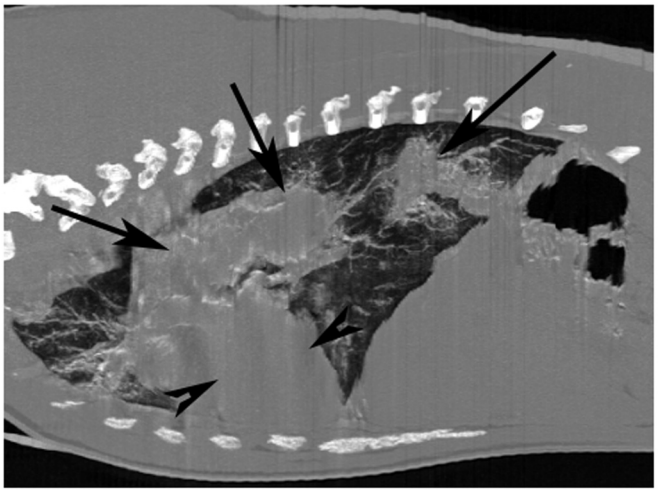

Fig. 1. Sagittal reconstructed CT image of the less severe alveolar pulmonary infiltrates in the left cranial and caudal lung lobes (black arrows). The linear streaking through the caudal thorax is beam hardening artifact secondary to incidental subcutaneous shot pellets. The black arrow heads are at the cranial and caudal margins of the heart.

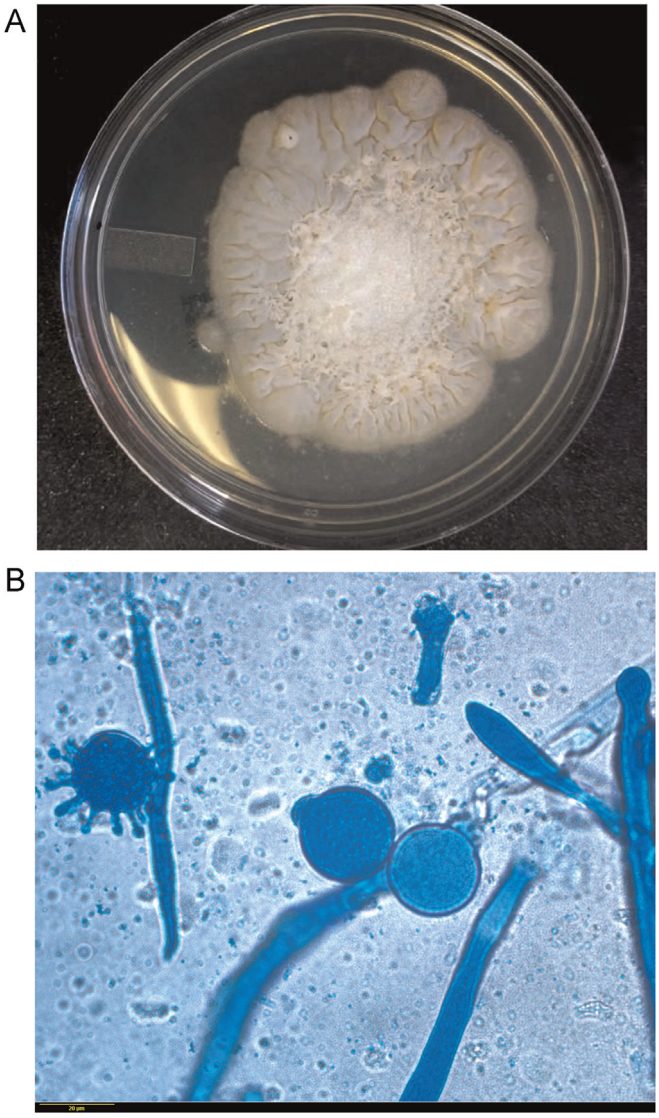

Fig. 2. (A) Two day culture of *Conidiobolus incongruus* on SDA at 37 °C (colony surface). (B) Multireplicative conidia, papillate conidia and hyaline coenocytic hyphae of *Conidiobolus incongruus*, in lactophenol cotton blue. Bar, 20 μm.

ME USA) test was negative (*Dirofilaria immitus* antigen and antibodies directed toward *Borrelia burgdorferi*, *Ehrlichia canis* and *Anaplasma phagocytophilum*). No intestinal parasites were seen on fecal floatation. Patient was treated with cefazolin (1200 mg IV every 8 h), enrofloxacin (162 mg IV every 24 h), gentamicin (350 mg IV every 24 h), guaifenesin (400 mg PO every 12 h) and intravenous fluids (30 mL/kg/day) for five days duration.

Seeing no improvement, patient was referred to OSU-BMVTH on day +11. Patient was depressed, dyspneic, and in poor body condition. Patient had lost 3.8 kg since day +7. A mild increased respiratory effort on inspiration and expiration. Lung sounds were harsh diffusely. Frequent productive cough was noted with expectoration of a white, foamy phlegm and occasional hemoptysis. The remainder of the physical examination was unremarkable.

Biochemical defects included hyperglobulinemia (4.9 g/dL; Reference range (RR): 1.6–3.6 g/dL) and hypoalbuminemia (2.4 g/dL; RR: 2.7–4.4 g/dL). Complete blood count showed mature neutrophilia (26,130/uL; RR: 2 060–10,600/uL), lymphopenia (670/uL; RR: 690–4500/uL), monocytosis (2 680/uL; RR: 0–840/uL) and eosinophilia (4 020/uL; RR: 0–1200/uL). Therapy consisted of intravenous fluids (60 mL/kg/day), nasal supplemental O_2 (1.5 L/min) ampicillin/sulbactam (22 mg/kg IV every 8 h), enrofloxacin (10 mg/kg IV every 24 h), doxycycline (4.7 mg/kg PO every 12 h), saline nebulization/coupage (every 6 h), and mirtazapine as an appetite stimulant (0.57 mg/kg PO every 24 h).

Diagnostics performed on day +12 included thoracic radiographs and bronchoscopy with bronchoalveolar lavage (BAL). Radiographic abnormalities included multifocal interstitial and alveolar pulmonary infiltrates in the right cranial, right middle and right caudal lung lobes. Bronchoscopic abnormalities include a raised, pink, irregular, proliferative lesion in the distal trachea, just proximal to the carina. A moderate amount of pink, foamy exudate was noted in the tracheal lumen. Opening to right cranial bronchus was obstructed with proliferative tissue and mucus. Right middle bronchus mucosa was mildly erythematous and also contains hemorrhagic mucus. BAL was performed and samples submitted for cytopathology, culture, PCR and *Histoplasma* antigen analysis. Cytopathology showed marked suppurative inflammation with evidence of previous hemorrhage. Aerobic bacterial culture and fungal culture were negative. Respiratory PCR panel was negative for canine influenza, adenovirus type-2, herpesvirus, coronavirus, H1N1, H5N1, canine distemper, *Bordetella bronchiseptica*, *Mycoplasma cynos* and *Streptococcus zooepidemicus*. BAL and urine *Histoplasma* antigen tests were negative.

Patient was prophylactically dewormed for parasitic pneumonia (fenbendazole 50 mg/kg PO every 24 h × 14 days, ivermectin 400 mcg/kg SQ once and praziquantel 10.9 mg/kg PO once) on day +12. Dexamethasone sodium phosphate (0.03 mg/kg IV every 24 h) was added on day +15.

Fungal serology (Idexx Laboratories, Westbrook, ME USA) for coccidiomycosis, blastomycosis, histoplasmosis, cryptococcosis and aspergillosis was negative.

Patient's clinical signs progressed from days +13 to +18. Repeat bronchoscopy was elected coupled with CT on day +19. That morning a firm, nodular cutaneous mass (1–2 cm) appeared on the rostral aspect of the patient's maxillary lip. Impressions cytopathology of this lesion were collected and submitted for review. CT abnormalities include multifocal alveolar infiltrates throughout the lungs. This finding was most severe in the right cranial and right middle lung lobes (Fig. 1).

Repeat bronchoscopy revealed multiple areas of erythematous and irregularly marginated proliferative tissue present from the distal aspect of the trachea both dorsally and ventrolaterally

Table 1
Reported cases of conidiobolomycosis from 2001–2014.

Organism	Species [Ref.]	Location	Year	#	Site	Diagnosis	Treatment	Result
Conidiobolus sp.	Dog [12]	USA	2006	1	Pulmonary	C	Itraconazole	Resolved
	Sheep [14]	Trinidad	2001	1	Rhinocerebral	H, C	None	Euthanasia
C. incongruus	White tailed deer [6]	USA	2009	1	Disseminated	H, PCR	None	Euthanasia
C. lampragues	Sheep [14]	Brazil	2010	9	Rhinocerebral	H, C, PCR	Unknown	Death or Euthanasia
	Sheep [11]	Brazil	2009	3	Nasopharyngeal	C, PCR	Unknown	Unknown
	Sheep [17]	Brazil	2006–2012	15	Nasopharyngeal	H, C(8), PCR(12)	Unknown	Death or Euthanasia
	Sheep [18]	Brazil	2006	6	Rhinocerebral	C, PCR (3)	Unknown	Death or Euthanasia
C. coranatus	Sheep [7]	Brazil	2007	60	Disseminated	H, C	None	Euthanasia
	Horse [19]	USA	2010	1	Nasal	H, C	None	Euthanasia
	Horse [20]	USA	2003	2	Nasopharyngeal	H, C	Fluconazole	Resolved

Table 2
Reported cases of conidiobolomycosis in the dog.

Org.	Species [Ref.]	Location	Year	#	Site	Diagnosis	Tx	Conc.
Conidiobolus sp.	Dog [12]	USA	2006	1	Pulmonary	C	Itraconazole	Resolved
	Dog [15]	USA	1993	1	Subcutaneous	H, C	Itraconazole	Euthanasia
	Dog [13]	USA	1996	1	Oral	H, C	Itraconazole	Resolved

Table 3
Reported cases of *C. incongruus*.

Org.	Species [Ref.]	Location	Year	#	Site	Diagnosis	Tx	Conc.
C. incongruous	Red deer [3]	Australia	1997	1	Disseminated	H, C	None	Death
	Sheep [5]	Australia	1987	5	Rhinocerebral (1) Nasal (4)	H (4), C (2)	None (4) Na Iodide (1)	Death (1) Euthanasia (4)
	Sheep [4]	Australia	1991	700	Nasal with hematogenous spread to thorax	H (4), C (4)	None	Death or Euthanasia
	White-tailed deer [6]	USA	2009	1	Disseminated	H, PCR	None	Euthanasia

Legend: H, histopathology; C, culture; PCR, polymerase chain reaction.

extending to the carina and mainstem bronchi, resulting in approximately 60% occlusion of the lumen. Hemorrhagic fluid was present throughout the trachea and bronchi. Significantly more proliferation was present as compared to initial bronchoscopy (day +12) preventing full examination. Tissue biopsy was submitted for histopathology. Impression cytopathology of maxillary nodule showed marked pyogranulomatous inflammation with fungal hyphae-like structures and eosinophilic infiltration. Biopsy showed marked suppurative to pyogranulomatous inflammation and epithelial atypia often seen with neoplasia.

Abdominal ultrasound was also performed on day +19. A small mass was present in the jejunal wall causing a complete loss of bowel wall layering within the central region of the mass. Within the more orad and aborad margins of mass, partial wall layering was present as the lesion transitioned into normal appearing bowel. The lesion was 50 mm (mm) in length. The thickest region of the mass measured 10 mm. The surrounding mesentery was hyperechoic, and a scant amount of regional hypoechoic free abdominal effusion was also present.

Owners elected euthanasia on day +20 due to lack of improvement and poor prognosis. Pre-mortem blood was collected for *Pythium* serology, which was negative. Patient was submitted to Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for necropsy.

On gross necropsy moderate, chronic, multifocal pulmonary nodules were seen. There was moderate, chronic, severe transmural thickening of the small intestinal wall with ulceration. The trachea was also characterized by marked, focal to coalescing nodular ulcerations and a chronic, focal, cutaneous ulcerated nodule was present on the face. The spleen demonstrated marked, disseminated lymphoid hyperplasia with capsular siderotic plaques, and there were multiple hepatic nodules of regenerative hyperplasia. Microscopic exam findings of the trachea, small intestine, and lesion on the maxilla showed pyogranulomatous inflammation, eosinophilic infiltrates, as well as intralesional fungi. Pyogranulomatous pneumonia was present in the lungs. Pulmonary intralesional structures partially consistent with fungal hyphae were noted and highlighted via silver staining, but the definitive identification as fungi consistent with other sites was lacking. It is likely that the pulmonary sites represented fungal pyogranulomas, but that their maturity and presumed role as a primary nidus of infection resulted in cell-mediated immune destruction of intact, recognizable hyphae.

3. Morphologic and molecular identification

Fungal culture was performed on the skin sample collected from the rostral aspect of the maxillary lip. Samples were inoculated onto Sabouraud dextrose agar (SDA, Hardy Diagnostics, Santa Monica, CA) culture plates. The plates were incubated at 37 °C and 25 °C. Fungal growth was detected after 24 h incubation

at 37 °C. Individual nascent colonies were transferred on to separate fresh SDA culture plates and incubated at 37 °C for pure growth and determination of colony characteristics. By Day 2, isolated colonies showed a flat glabrous appearance typical of a *Conidiobolus* sp. (Fig. 2A). The colonies initially appeared white in color which later changed to tan with age. Microscopically they were characterized by coenocytic hyaline hyphae, numerous papillate conidia (10–35 μm) and a few multireplicative conidia (Fig. 2B).

This isolate was sent to the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center at San Antonio (UTHSCSA DI 14-347) for species level identification by combined phenotypic characterization and DNA sequencing. The isolate that was received was non-viable. However, photomicrographs that had been taken by the requesting laboratory (OADDL) demonstrated multi-replicative conidia (Fig. 2B). DNA sequencing on the non-viable isolate was attempted by scraping the agar surface and suspending the fungal elements in CPL-100 Buffer (VWR International INC., Radnor, PA). These were then lysed by bead beating, and DNA was isolated manually by chloroform extraction method. Extracted DNA was used for PCR amplification of ITS and D1/D2 regions as described in [9]. PCR products were then sequenced using the ITS1 and ITS4 primers as well as NL1 and NL4 primers at the UTHSCSA Molecular Diagnostics Laboratory [10]. Sequences were assembled and analyzed using DNASTAR software (DNASTAR, Inc., Madison, WI) and queried in GenBank using the BLASTn algorithm at the NCBI site (www.ncbi.nlm.nih.gov), and were also compared to those available in the CBS-KNAW Fungal Biodiversity Centre database (www.cbs.knaw.nl). Sequencing of the ITS region was unsuccessful. However, the D1/D2 sequence showed 98.9% identity to *C. incongruus* NRRL 28636 (GenBank Accession no. AF113457.1; base pair match 617/624). Based on the presence of the multi-replicative conidia, which have been described as a discriminating characteristic between *C. incongruus* and *C. lamprauges* [11], and the D1/D2 sequence analysis, the species of this isolate was determined to be *C. incongruus*. The D1/D2 sequence has been deposited in GenBank under Accession no. KP777609.

4. Discussion

Conidiobolus species are members of the Order *Entomophthorales*, Subphylum *Entomophthoromycotina,* Class *Glomeromycetes* and the Phylum *Glomeromycota* (formerly Zygomycetes and Zygomycota) [1]. These fungi are saprobes, often found in decaying plant matter and soil, and can also exist as facultative or obligate insect pathogens [1,2,12]. *Conidiobolus* infections are distributed worldwide, predominantly in tropical and subtropical climates, and are most often found in immunocompetent patients [1,2].

Several species of *Conidiobolus* have been reported to cause disease in both humans and animals. These species include *C. coronatus*, *C. incongruus*, and *C. lamprauges.* Conidiobolomycosis is usually a localized infection that is most commonly caused by *C. coronatus* [1]. Clinically, the infection manifests as a nasopharyngeal disease that can extend to include the tissues of the face, retropharyngeal region, retrobulbar space and regional lymph nodes. Patients may present with chronic nasal disease, multi-focal nodular subcutaneous lesions, ulcerative dermatitis or, less commonly, pneumonia [1–8, 12–17].

Evidence based treatment protocols for conidiobolomycosis are lacking. This is due to both the low number of reported cases as well as the fact that patients often present in the late stage of disease when therapy is unrewarding. Complete and aggressive surgical excision is considered the gold standard treatment; however, the location of infective tissues may preclude surgical intervention. Many different antifungals, such as amphotericin B, ketoconazole, itraconazole and potassium iodide, have been used alone or in combination with marginal success [2,5,12,13,15,20]. Long-term therapy is recommended for several months and recurrence is common.

To the best of our knowledge, this is the first report of *C. incongruus* isolated from the dog. There have been previous reports of *Conidiobolus* spp. in dogs, but the particular species was not identified [12,15]. Several other cases and outbreaks of conidiobolomycosis have been reported in animals and are summarized in Tables 1–3.

Primary pulmonary infection is a rare presentation regardless of species [12]. While nasopharyngeal disease is typical, infection of the deeper airways is uncommon. Tracheal involvement has been reported in one dog and one horse, neither of whom had pulmonary or nasopharyngeal disease [12]. This case is also one of only two reported canine cases of fungal pneumonia secondary to conidiobolomycosis [12]. The previous case reported by Hawkins, et. al. also occurred in a dog and the isolate was identified as a *Conidiobolus* sp. Due to its morphology *C. coronatus* was ruled out, but further identification was not possible [12]. The dog was successfully treated with itraconazole; however disseminated disease was not present. In our case the lungs were the initial site of infection with dissemination to the skin of the face, trachea as well as both small and large intestines.

Further specific treatments with antifungals were not attempted in this dog because of the extent and severity of the lesions, as well as the sharp clinical demise. Moreover, tracheal biopsy tissue displayed marked epithelial atypia, and a neoplastic process was still under consideration, which contributed to the grave prognosis. With the benefit of comprehensive necropsy and histology of many organs, including the tracheal site of interest, the epithelial atypia was determined to be consistent with reactive tissue within a severely inflamed focus. Nevertheless, specific antifungal therapies could not be tested in this unique case, and it is unknown how the dog would have responded to that therapy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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