Network for wildlife health surveillance in Europe

Diagnosis Card

Ophidiomycosis – Snake Fungal Disease (SFD)

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Etiology

Ophidiomycosis, also known as Snake Fungal Disease (SFD), is a fungal disease caused by *Ophidiomyces ophidiicola* (Oo), an ascomycete fungus belonging to the order Onygenales. Three clades (I "European clade", II "North American clade", III) are supported by different phylogenetic analyses, but their geographic range, origin, and variation in strain virulence remains unknown. Two mating types of Oo have been reported.

Affected species (wildlife, domestic animals, humans)

Oo has been detected in >65 species and 9 families of snakes. The majority of these were diagnosed as confirmed ophidiomycosis (histology + molecular detection) or were apparently affected by the disease (gross signs + molecular detection). The known host spectrum and diversity of affected snake species continues to expand, particularly in temperate regions of the Northern Hemisphere. Semi-aquatic species are more likely to become infected with Oo, i.e., species from family Natricidae such as *Natrix* spp. in Europe and *Nerodia* spp. in North America. Oo is capable of infecting lizards based on an experimental trial, but naturally occurring infections in reptiles other than snakes have not yet been reported. The disease has been reported in captive ophidians across multiple continents in recent decades. Captive reptiles and their trade might have a role in past spillover events of Oo ("pathogen pollution"). Museum specimen testing has detected Oo in wild snakes in both Europe and North America dating back to the 1950s. Genetic studies support multiple recent introductions of Oo into North America.

Epidemiological characteristics and disease course

The occurrence of ophidiomycosis appears to vary by season, with the overwintering period, low temperatures, and contaminated hibernacula potentially playing key roles in the pathogenesis of the disease and transmission of the pathogen. In most of the populations affected in temperate zones, the peak of disease manifestation is during the peri-brumation periods. A seasonal pattern of disease is likely to be driven by a vicious cycle in which suboptimal immune function, increased stress response (lower temperatures, higher cortisol, testosterone, and estradiol levels) and lower resource acquisition act synergistically to increase host susceptibility, similarly to what has been shown in other reptiles. Hibernacula are considered potential natural reservoirs of Oo. Accordingly, the overwintering of snakes in these sites might contribute to the colonization of snakes by the fungus. However, soil microbiota might significantly affect the abundance of the fungus in the environment. In addition to exposure from contaminated environments, transmission also likely occurs due to snake-to-snake contact, including transmission from female snakes to their young. Fungal infection and subsequent development of ophidiomycosis in snakes does not require the presence of open wounds or damaged skin; however, epidermal ulcerations and lacerations may increase the likelihood of infection. Commonly, Oo infection leads to variably severe skin disease (dermatitis), and, more rarely, systemic spread. According to the severity and distribution (localized versus systemic) of the infection, the disease may naturally resolve (e.g., via increased frequency of ecdysis) or result in mortality.

Clinical signs

Oo infection can range from subclinical to clinical and may, in some instances, be fatal. Cutaneous

lesions are the most frequently reported clinical occurrence. Similar to other reptilian taxa affected by infectious diseases, infected snakes tend to thermoregulate at relatively high temperatures, presumably to increase the efficacy of their immune response. Accordingly, diseased individuals might be observed basking at unusual times and in open spaces, significantly increasing the risk of being predated or dying of exposure. Severely affected ophidians might display poor body condition and lethargy, with associated reduction of visceral adipose tissue, indicating a secondary energetic deficit, often part of a general deterioration that may eventually lead to death.

Gross lesions

Skin lesions are usually represented by multifocal to coalescing areas where the scales are deformed, alternatively indented, thickened, raised, and necrotic. Affected scales are typically covered in light tan to brown crusts. During certain stages of infection, affected scales may appear whitened and edematous. Erosion of necrotic tissue may also result in exposure of the deeper layers of epidermis and dermis. When the head is affected, edema and crusting of the skin can cause disfiguration of the head and damage to the eyes. Affected scales may remain deformed even after the infection has resolved.
Deeper infections reaching the subcutis and underlying skeletal muscle may be associated with the development of nodules. In extremely severe cases, the infection can become systemic, affecting potentially any visceral organ.

Histological lesions

Infections are often limited to the epidermis and characterized by the presence of serocellular crusts, hyperkeratosis, extra and intracellular edema. The dermis may be variably infiltrated by inflammatory cells along with occurrence of edema and fibrosis in more chronic cases. Severe dermatitis characterized by the occurrence of heterophilic granulomas often encasing fungal hyphae are a relatively common finding. Infection of the dermis, hypodermal mycetomas, and invasion into the musculature immediately underneath the skin may also occur. There have been reports of infection in deeper tissues such as the air sacs, bronchi, lungs, trachea, esophagus, stomach, mesentery, gingiva, salivary glands, eyes, coelomic fat, ovaries, kidneys, liver, and spleen. Intralesional hyphae are transversally septate, have parallel walls, up to 5 µm in diameter, with frequent acute-angle branching. Arthroconidia are approximately 2 × 4 µm, cylindrical, and can be observed intralesionally either in fission formation (separating from fertile hyphae) and/or in compact clusters (arthroconidial tufts) at the air-tissue interface. In addition, bullet-shaped aleurioconidia may sometimes be present along the surface of the skin.

Differential diagnosis

Other fungal dermatitis are caused by *Paranannizziopsis* spp., *Nannizziopsis* spp., *Geotrichum* spp., *Trichophyton* spp., *Fusarium* spp. Bacterial dermatitis. Viral dermatitis. Necrosis of the epidermis due to trauma or other injury may be confused with clinical signs of ophidiomycosis.

Criteria for diagnosis

Criteria for case definitions have been developed as follows:

- Oo detection (PCR) or culture) and histopathological presence of fungal hyphae (with or without conidia) consistent with Oo in association with microscopic lesions (*conclusive* ophidiomycosis)
- Oo detection (PCR or culture) in association with gross lesions (*apparent/presumptive* ophidiomycosis)
- Gross lesions and histologic evidence of fungal hyphae (especially with conidia) consistent with Oo with negative or doubtful Oo detection (*suspected* ophidiomycosis)
- Oo detection (PCR or culture) with no detected gross lesions (Oo *detected*)

Due to the ability of Oo to cause subclinical infections and for morphologically similar fungi (e.g., *Paranannizziopsis* spp., *Nannizziopsis* spp.) to cause skin lesions in snakes, it is possible for Oo to be detected without it being the cause of disease. Therefore, it is advisable to be cautious when interpreting low-level molecular detections of Oo in snakes with skin lesions and to consider screening for other possible etiologies.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Initial Oo targeted detection: conventional polymerase chain reaction (PCR) or real time PCR (qPCR) with primers based on specific DNA sequences for Oo within the internal transcribed spacer 1 or 2 regions (ITS1, ITS2) or intergenic spacer region (IGS) within the ribosomal RNA (rRNA) gene complex. qPCR targeting the ITS2 region is the most widely used method and is generally considered the gold standard for detection of Oo.
Initial panfungal screening: Broad-range panfungal PCR targeting the D1-D2 region or the ITS region of the large subunit of the rRNA gene (note that follow up sequencing of amplicons is necessary for identification). This method may be useful when a fungal etiology has been identified by other means

(e.g., histopathology) and initial Oo targeted detection has failed or is unavailable. Caution is needed when interpreting negative results as mixed fungal populations may obscure the presence of Oo. Panfungal PCR methods are more useful for confirming the identification of isolates obtained in culture. *Characterization of positive samples*: limited genotyping of strains can be accomplished through amplification and amplicon sequencing of multiple chromosomal loci (ITS and partial sequences of the actin [ACT] and translation elongation factor 1-α [TEF] genes) from both clinical samples and isolates. *Isolation*: culture on Sabouraud dextrose agar (SDA) or inhibitory mould agar (ICG) with chloramphenicol and gentamicin at 22–25°C for 10–20 days. Selective cultivation of samples on dermatophyte test medium (DTM) or Mycosel agar at 30 °C for 10–20 days often results in a higher success rate for isolation of Oo when samples are likely to be contaminated with non-target fungi. *Samples of choice*: tissues or skin swabs for molecular detection; tissues (preferable) and moistened swabs (distilled water or PBS) for isolation. Samples can either be analyzed fresh or stored at -20 to -80°C until analysis. *Histology*: on tissue samples fixed in 10% neutral-buffered formalin (NBF) and embedded in paraffin. Sections stained with periodic acid–Schiff (PAS) or silver stains (e.g. Gomori's, Grocott's) to highlight fungal elements. Tissue samples of choice: skin, preferably including all layers of the skin. When systemic spread is suspected, lung, liver, or any tissues with gross lesions observed during necropsy can also be considered. No routine immunohistochemistry or in situ hybridization are currently available; however, experimental in situ hybridization protocols have been developed.

EWDA proposed harmonized protocol (for harmonization at large scale)

Real time PCR of ITS2 region and histology/fungal isolation on positive samples. The above-mentioned samples must be transported to the laboratory refrigerated in multiple aliquots, of which one (tissue) is fixed in 10% neutral-buffered formalin (histology), and the others (tissues or swabs) either analyzed for qPCR/fungus culture immediately or samples stored at -20 to -80°C until analysis.

Laboratories that can be contacted for diagnostic support).

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- United States, U.S. Geological Survey – National Wildlife Health Center (jlorch@usgs.gov) (*any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government*)

Recommended literature

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