SEPARC DTT
Information Sheet #21

Snake Fungal Disease (Ophidiomycosis) in Southeastern Snake Populations

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Clinical Signs of SFD

The most prominent clinical signs of SFD are the presence of heterophilic granulomas, crusty dermal lesions, deformed scales, swelling of the head, and cutaneous ulcers (Allender et al., 2015; Lorch et al., 2015; See Figure 1). Clinical signs which are non-specific and are unlikely to be diagnostic in a field setting, but which are associated with SFD, include lethargy and an increased rate of ecdysis (Lorch et al., 2015). Lesions resulting from infection with *O. ophiodiicola c*an be present anywhere on the snake's body with the development of severe lesions typically occurring around the face, eyes, nostrils, and, in the case of pit vipers, the loreal pits. While SFD lesions are usually associated with the dermis, severe infections can penetrate into muscle and bone (Lorch et al., 2016).

Figure 1. A female *Nerodia sipedon* exhibiting characteristic crusty brown and deformed scales associated with ophidiomycosis on the head and tail. Photo credit Cody Davis Godwin.

Pathogen Characteristics

Snake Fungal Disease (SFD) is caused by the fungus *Ophidiomyces ophiodiicola* (Allender et al., 2015; Lorch et al., 2015); *O. ophiodiicola* is a member of the order Onygenales which contains many keratinophilic species of fungi and human pathogens of clinical significance (Koufopanou et al., 2001; Paré and Sigler 2016; Sharma and Rajak, 2003). *Ophidiomyces ophiodiicola* was originally classified within the genus *Chrysosporium*; which contains other cutaneous pathogens of reptiles (Paré and Sigler, 2016); however, phylogenetic analysis of sequence data from the nuclear rDNA ITS and 18S regions led to the description of a new genus to describe *O. ophiodiicola* (Sigler and Paré, 2013). Several hypotheses have been proposed to explain transmission of SFD including direct contact between uninfected and infected individuals and/or inoculation from environmental reservoirs (Lorch et al., 2016). It has been suggested that *O. ophiodiicola* may persist as a saprotroph in soil due to its ability to utilize a wide variety of carbon and nitrogen sources, tolerate a wide range of environmental pH and temperatures, and endure water stress (Allender et al., 2015; Rajeev et al., 2009). In at least one study to date, the molecular presence of *O. ophiodiicola* has been detected on the landscape from soil samples lending support to this hypothesis (Walker et al., 2019). Dermal abrasions and exposed soft tissue are hypothesized avenues for infection into the host body but may not be necessary to establish infections (Allender et al., 2015; Lorch et al., 2015). Recent work has suggested brumation may play a role in horizontal transmission of *O. ophiodiicola* (McKenzie et al., 2020). Additionally, vertical transmission of *O. ophiodiicola* from mother to offspring has been documented (Stengle et al., 2019). Once established on the epidermis, fungal hyphae penetrate into the skin of snakes and can reach underlying muscle and bone (Lorch et al., 2015). However, *O. ophiodiicola* is also reported to persist on snakes with no apparent clinical disease (lack of granulomas or deformed scales) (Pare et al., 2013; Bohuski et al., 2015). As of 2020, SFD has been discovered in 38 U.S. states and Puerto Rico (Allender et al., 2019). Additionally, SFD has been documented in Europe (Franklinos et al., 2017) in captive snakes in Australia (Sigler et al., 2013), and recently in Taiwan (Sun et al., 2021) and Japan (Takami et al., 2020). Snake Fungal Disease has been reported in 15 genera of wild and captive snakes, and some modeling efforts indicate that hosts may be ecologically and phylogenetically random (Burbrink et al., 2017; Allender et al., 2019). However, more recent work suggests that there are taxonomic associations with SFD epidemiology such that some genera have higher rates of apparent infection (Haynes et al., 2020).

Diagnosing SFD

A combination of diagnostic methods is suggested for successful SFD diagnosis (McKenzie et al., 2019). Currently there are four accepted methods for SFD detection: 1) diagnosis through clinical signs, 2) use of dermal swabs to detect *O. ophiodiicola* DNA, 3)

histopathology of infected tissue, and 4) culturing the fungus from infected tissue. Visible granulomas and crusty lesions are considered to be characteristic of SFD infections, but deformed scales may simply be scar tissue generated by any number of non-SFD related processes. The use of dermal swabs to identify *O. ophiodiicola* DNA via PCR/qPCR is an effective method for detecting the causative agent of SFD. However, the risk of false negatives with dermal swabs can be high so multiple samples should be taken and assayed (Hileman et al., 2018). Additionally, snakes may test positive for the molecular presence of the pathogen but lack clinical signs. Histopathology of infected tissue is another viable option for detecting *O. ophiodiicola* hyphae but requires tissue collection either through a scale clip or biopsy punch. Additionally, this method is time consuming, and detection of fungal hyphae can be low. Finally, culturing *O. ophiodiicola* from suspected skin lesions is an option for diagnosis of snakes with SFD. Culturing *O. ophiodiicola* can be challenging and initial attempts to culture *O. ophiodiicola* lead to descriptions of opportunistic fungal taxa (McBride et al., 2015). Baker et al. (2019) has created a diagnostic protocol that allows for successful classification of SFD diagnoses with a combination of the previously mentioned detection methods (Table 1.)

Table 1. Representation of the diagnostic results that correspond with each of the diagnostic categories presented by Baker et al., (2019).

Diagnostic Category	Detection of Clinical Signs	Histopathology Results	qPCR Assay Results	Culturing Success
Negative	-	-	-	-
Ophidiomyces Present	-	-	+/-	+/-
Possible Ophidiomycosis	+/-	+/-	-	-
Apparent Ophidiomycosis	+	-	+	-
Confirmed Ophidiomycosis	+/-	+	+	+

Snake Mortality Events:

Currently, SFD is not broadly implicated in extirpation or die-off events within snake populations. The most well documented declines of snake populations resulting from SFD involved two species of American pit vipers. In 2006, individuals of the last known

population of timber rattlesnakes (*Crotalus horridus*) in New Hampshire became afflicted with a fungal pathogen that caused severe, and often lethal, infections. The authors of this paper suggested that a myriad of factors such as reduced genetic diversity, the impacts of climate change, and this unknown fungal pathogen contributed to the overall decline and extirpation of this rattlesnake population (Clark et al., 2011). While this event has been cited as the first documented outbreak of SFD (Allender et al., 2015), the lack of molecular or microscopic identification of the etiologic agent has made it impossible to conclusively confirm this as an occurrence of SFD (Clark et al., 2011). In 2008, a fungal pathogen was reported among members of an Illinois population of eastern massasauga rattlesnakes (*Sistrurus catenatus*) which resulted in facial swelling and mortality (Allender et al. 2011).

Although no similar event has been reported in the southeastern United States, it must be noted that sampling bias is a significant confounding variable in the study of free-ranging snake populations. Snakes are notoriously cryptic (Lukoschek, 2018). Additionally, they do not typically congregate in large groups with the exception of some pit viper and *Thamnophis* species during brumation and reproduction (Joy and Crews, 1985; Reinert and Zappalorti, 1988). As a result, it can be difficult to generate reliable estimates of population dynamics, juvenile recruitment, disease prevalence and mortality in snake populations (Valenzuela-Sánchez et al., 2017; Wilson et al., 2011; Reading et al., 2010).

Determining the impact of SFD on snake populations requires monitoring programs with a broad spatial and temporal scope. Because monitoring programs are rare, the relative scarcity of snake declines attributed to SFD may be due to inadequate sampling effort. Snakes exhibiting severe lesions of the face, eyes, and nostrils are believed to have reduced foraging success and perish slowly from starvation (Lorch et al., 2016). It is likely severe *O. ophiodiicola* lesions allow for secondary infections and sepsis. Also, snakes with severe infections are more prone to bask earlier in the year and may die from increased exposure to predation or the elements (Lorch et al., 2015). Sublethal consequences associated with *O. ophiodiicola* infections have been noted. For example, infection with *O. ophiodiicola* in a population of free-ranging pygmy rattlesnakes (*Sistrurus miliarius*) was correlated with increased resting metabolic rate and evaporative water loss (Agugliaro et al., 2019). In another study, of the same population of pygmy rattlesnakes, SFD was associated with lower levels of testosterone in males during spermatogenesis and breeding potentially resulting in depression of reproductive success (Lind et al., 2019).

Factors Contributing to Emergence

Research suggests that *O. ophiodiicola* is not a novel pathogen to North America but may have been endemic before formal description. Historically, herpetologists simply referred to similar lesions as “hibernation sores.” This is due to the fact that snakes emerging from hibernation frequently exhibited lesions which are now considered

characteristic of SFD (Lorch et al., 2016). This anecdotal evidence does not support the hypothesis that *O. ophiodiicola* is native to the United States in and of itself, and any number of diseases could have resulted in historic observations of "hibernation sores", but it is possible snakes exhibiting "hibernation sores" had *O. ophiodiicola* infections." Lack of detection would have likely been due to misdiagnosis and limited snake monitoring programs (Lorch et al., 2016). Analysis of skin lesions from preserved museum specimens suggests *O. ophiodiicola* may have been present in the United States for decades (Cheatwood et al., 2003; Lorch et al., 2016). Additionally, the current distribution of SFD detection does not follow a typical pattern associated with an introduced pathogen where an origin site is detected and a nonrandom geographical pattern exists radiating from the origin. The eastern and mid-western United States seems to be the origin of the pathogen.

Climate change may facilitate spread and growth of *O. ophiodiicola* if it expands thermal and humidity ranges conducive for *O. ophiodiicola* survivorship (Lorch et al., 2016). Earlier studies suggest increased rainfall and wetter conditions allowed for increased survivorship of a previously undescribed snake fungal pathogen (Clark et al., 2011). Climate change also may affect temperatures during hibernation that allow successful growth of *O. ophiodiicola* on its host (Allender et al., 2015)

Reduced genetic diversity in isolated populations also may contribute to SFD prevalence and severity via suppressed immune response. Reduced genetic diversity, climate change, and presence of a fungal pathogen were considered synergistic in the decline of timber rattlesnakes in New Hampshire (Clark et al., 2011).

Preventative Measures

Experimental treatments using nebulized and subcutaneously implanted terbinafine have shown promise in treating snakes suffering from SFD (Kane et al., 2017; Guzman-Vargas et al., 2020). However, these treatments are unlikely to be useful as a large-scale management strategy due to their requirement for individual-level care and removal of infected individuals from the field. Reduction of the accidental spread of SFD from the activities of field biologists is likely the most feasible and affordable mitigation strategy to date. It is imperative to not move snakes or introduce snakes to an area outside of their dispersal range. Reintroductions for reestablishment of snake populations are an exception, but only after thorough health screenings that incorporate use of qPCR assays and visual inspections for SFD. Snakes can be asymptomatic but still carry *O. ophiodiicola* (Pare et al., 2013; Bohuski et al., 2015); therefore, moving apparently healthy snakes can pose a risk for spreading of the pathogen. Field biologists and herpetologists should be cognizant of SFD and good equipment hygiene is paramount. Table 2 provides a comprehensive list of effective disinfectants that can be used to neutralize *O. ophiodiicola* on a variety of surfaces. Footwear should be cleaned of debris and sprayed with a 3% bleach solution before and after entering the field. Snake hooks, tongs, tubes, and any other hard equipment

should be sprayed down with bleach solution after contact with snakes in the wild and between handling different snakes to reduce chances of accidental transmission from infected to healthy snakes. The 3% bleach solution can be rinsed off of equipment after a 2-5 minute contact time. The use of latex or nitrile gloves is recommended when handling snakes and exchanging gloves between snakes can reduce the chance of possible transmission. Active monitoring programs and studies of wild snake populations should incorporate testing for SFD and good biosecurity protocols.

Table 2. Effective disinfectants, concentrations, contact time, and applications to neutralize *O. ophiodiicola*. Information derived from Rzadkowska et al., (2016) and Gray et al., (2017).

Disinfectant	Concentration	Contact Time	Application
Sodium Hypochlorite (Bleach)	3%	2-5 min	Surfaces except plastics
Virkon	1%	2-10 min	Safe on all surfaces
Phenols (Lysol™)	2-5%	10 min	Hard surfaces must be rinsed
Ethanol	70%	2-10 min	Surgical equipment, Hands, and countertops
Benzalkonium Chloride	0.1%-0.2%	1 min	Surgical equipment
CLR™		10+ min	Hard surfaces must be rinsed
Quaternary Ammonium Compounds	0.4%	10+ min	Spray or dip equipment must be rinsed off

What to do if SFD is suspected?

Snakes exhibiting lesions consistent with SFD should be reported to the PARC Disease Task Team at herp_disease_alert@parcplace.org. In your email, please include the following information: your name and email address to allow for follow-up questions, date of the observation, a detailed description of what you saw, where the observation occurred, what animals were involved (species and life stage), outbreak status or prevalence (i.e. how many animals were involved, what was the severity of their signs, were there mortalities), and any photos you collected or other relevant information. This information will be shared with the state conservation agency or other relevant authority for the area where the concern was documented.

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