NEW AND EMERGING FUNGAL PATHOGENS IN MICHIGAN AND THEIR DIAGNOSIS

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Introduction

Emerging fungal pathogens are an increasing global threat to exotic pets, zoo and wild animals. The global spread of these diseases is thought to be facilitated by climate change and anthropogenic activities including global trade of captive wild animals. In the last 10 to 15 years there have been reports of several new and emerging fungal diseases affecting wild and captive wild animals in North America. These include the white nose syndrome caused by Pseudogymnoascus destructans, a devastating disease of hibernating bats which has led to mass mortality in bats, chytrid fungal disease affecting endemic and captive amphibians, wiping out more than 200 species of frogs worldwide, and the Chrysosporium anamorph Nannizziopsis vriesii (CANV) complex group of fungi affecting reptiles. The CANV group includes Chrysosporium vriesii and C. guarroi which causes deep dermal mycosis in reptiles and Ophidiomyces ophiodiicola which causes snake fungal disease. The State of Michigan has also seen the emergence of these fungal diseases. White nose syndrome has affected the little brown bat population in Michigan while the snake fungal disease is currently a threat to the eastern massasauga rattlesnake in the state. Some of these diseases are considered as newly introduced while others may be considered as emerging. Geographic distribution and progression of the white nose syndrome and genetic typing of P. destructans has revealed that this pathogen is novel to North America and was introduced recently, possibly by human activity. The chytridiomycosis panzootics, first reported in 1998 can also be considered as a newly introduced disease. Although the etiologic agent Batrachochytrium dendrobatidis has been known to infect amphibians previously, the recent panzootics is believed to be due to a highly virulent strain of the fungus. The CANV group of fungi which include O. ophidiicola and Nannizziopsis vriesii group are saprophytes found in the environment and are emerging pathogens of reptiles with their origin and virulence still being investigated. The diagnostic methods for detection of these new and emerging fungal pathogens include histopathology, culture, and molecular techniques including PCR and MALDI-TOF MS technology. Increased awareness of these dangerous infectious diseases may help in their early diagnosis and management.

White Nose Syndrome in Bats

The first report of white nose syndrome (WNS) in bats in North America was from Albany, New York in 2006. Thereafter the disease has spread geographically southward and westward and currently the disease has established itself in eastern half of the United States and eastern Canada. European bats have been shown to be tolerant to the etiological agent of WNS namely P. destructans. The continued geographical progression of the disease in North America and lack of genetic diversity among the fungal isolates and European strains
indicates the recent introduction of this fungus to immunologically naïve native bats in the continent. In Michigan WNS was first reported from Alpena County in 2014. Currently the disease has spread to two additional counties in the Lower Peninsula and seven counties in the Upper Peninsula. *Psuedogymnoascus destructans* is a psychrophilic fungus and the bats are especially susceptible to infection during their hibernation with the most prominent sign of infection being the cottony white growth of the fungus on the snouts of these bats. During hibernation the bats are thought to be in an immunologically dormant state and signs of inflammatory response to the fungus in the infected bats are absent. The frequent interruption from the hibernation as a result of this infection leads to energy depletion, starvation and death. The observation of fluorescence of the snout, tail and wing membranes under ultraviolet light for the diagnosis is used but may result in false diagnosis. Confirmatory diagnosis can be achieved by combination of histopathology, PCR and or culture. Because of the psychrophilic nature of this fungus and high probability of contamination from clinical samples culturing of *P. destructans* is challenging.

### Chytridiomycosis in Amphibians

Chytridiomycosis is now considered a panzootic affecting amphibians. In the last 20 years hundreds of frog species have been wiped out throughout the world. Although the chytrid fungus, *Batrachochytrium dendrobatidis* is a major culprit behind this decrease in amphibian population other factors including habitat destruction and pollution has also played a role in this drastic decline. The *B. dendrobatidis* is widespread in the United States and is thought to be spread by the invasive North American bullfrog which is resistant to the fungus and act as a reservoir for the pathogen. The chytrid fungus is found in soil and water and can infect the keratinous outer layer of frog skin. It is thought that fungal infection of the frog skin can lead to an electrolyte imbalance and cardiac arrest. The susceptible species of amphibians elicit little immunological response to the infection. Data relating to prevalence of this disease in the local amphibian population in Michigan is lacking; however, there have been reports of the disease in zoos and incidences related to pet trade in the state. Real time quantitative PCR is available for detection and quantifying *B. dendrobatidis* from clinical samples. Management of this disease includes treatment with fungicide dips and conservation efforts to restore endangered species by captive breeding. Research on amphibian antimicrobial peptides and other biological control methods on combatting the disease are promising.

### *Chrysosporium* anamorph Nannizziopsis vriesii (CANV) complex

*Nannizziopsis* genus

This fungal genus contains *N. vriesii* and *N. guarroi*, both are pathogens of wild and captive reptiles. They are considered emerging fungal pathogens and can cause deep dermal mycosis and sometimes systemic infections in bearded dragons, green iguanas, geckos, terrestrial and aquatic snakes and crocodiles. Fungal etiology is not always a top differential diagnosis in reptiles as they are often considered as contaminants but CANV is now recognized as an obligate pathogen of reptiles. Diagnosis can be confirmed by histopathology, PCR or culture. *Nannizziopsis* spp. are easy to culture and can grow on culture media relatively quickly (5-10 days). However morphology of the fungus alone is not sufficient to differentiate between several species of *Chrysosporium*, so secondary confirmation by fungal ribosomal DNA sequencing or MALDI-TOF MS has to be performed. The antifungal of choice for treatment is itraconazole and voriconazole, the latter has been frequently used due to its lower incidence of side effects.
Snake Fungal Disease (SFD).

This is an emerging fungal disease of North American endemic and captive snakes. The fungal etiological agent was previously classified under the genus *Chrysosporium* but is now classified under the genus *Ophidiomyces*. *Ophidiomyces ophiodiicola* persists in the environment as a saprobe in soil as well as on susceptible host species. Although the fungus was reported to be associated with disease in snakes previously, it was first reported as a pathogen of snakes in 2009. Currently the *O. ophiodiicola* has been reported to cause disease in free ranging and captive snakes in several of the eastern and midwestern states in the United States including Michigan. The SFD is a threat to the only venomous snake in Michigan, the eastern massasauga rattlesnake. The US Fish and Wildlife Service has recently added the eastern massasauga rattlesnake as a threatened species. Infection can vary from mild skin lesions with pustules and nodules to severe swelling and sometimes systemic infection involving multiple organs including muscle, bone, lung and liver. Ideal noninvasive method of diagnosis is PCR from skin lesions. *Ophidiomyces ophiodiicola* can colonize the skin of snakes without showing any clinical signs and therefore, confirmatory diagnosis of the disease has to be supported by histopathology. It has been recently reported the disease can be diagnosed by a quantitative real-time PCR as the presence of this disease correlates with the amount of fungal DNA recovered. Isolation of the fungus from skin lesions can also be considered confirmatory. The fungus can grow on selective media in 5-10 days and confirmatory diagnosis may be achieved by sequencing or MALDI-TOF MS. A combination of topical or systemic antifungals and supportive therapy with fluid, nutritional supplementation and surgical debridement are used for treatment. However for free ranging snakes treatment with antifungals has so far proven unsuccessful.

**Conclusion**

New and emerging fungal pathogens are not just limited to the above described select list of agents. Other fungal pathogens that are worth mentioning include the *Ascosphaera apis* and *Batrachochytrium slamandrivorans* threatening the bees and salamanders respectively. Together these emerging fungal diseases are devastating the endemic wild life, destroying the biodiversity and in turn upsetting the ecological balance. Although the emergence of many of these diseases can be traced back to anthropogenic activities, the influence of other factors including the emergence of virulent strains and climate change is still being elucidated. Continued increase in awareness and surveillance together with improved diagnostic methods and management of emerging fungal diseases are essential to prevent future outbreaks.

**References**

