Update on Snake Fungal Disease in Eastern Virginia

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Introduction:

Snake Fungal Disease (SFD) is an emerging wildlife disease caused by the fungus *Ophidiomyces ophiodiicola* (Oo) (Allender et al., 2015a; Lorch et al., 2015). SFD has been documented in a variety of snake species throughout the eastern and midwestern United States over the past ten years (Allender et al., 2016b). This disease is characterized by crusty scales, superficial pustules, subcutaneous nodules of the skin, dysecdysis, and ocular cloudiness with variable morbidity and mortality in snakes (Guthrie et al., 2016). Some species of snake including the Timber Rattlesnake (*Crotalus horridus*) and the Eastern Massasauga (*Sistrurus catenatus*) experience significant facial disfiguration and high mortality associated with SFD infection (Sutherland et al., 2014; Allender et al., 2015a). While the disease is mostly commonly associated with dermatomycosis, disseminated systemic infections have been documented in some snakes (Dolinski et al., 2014; Robertson et al., 2016). SFD has been documented in both captive and free-ranging snakes and has broad geographic and taxonomic distributions (Allender et al., 2016a).

In 2014, we conducted a study in southeastern Virginia; 30 free ranging non-venomous snakes were examined and eight of those snakes were positive for SFD using fungal culture, histopathology, and PCR testing (Guthrie et al., 2016). Species of snakes that were SFD positive in the 2014 study included the Brown Watersnake (*Nerodia taxispilota*), Common Rainbow Snake (*Farancia e. erytrogramma*), Northern Watersnake (*Nerodia s. sipedon*), Eastern Black Racer (*Coluber c. constrictor*), and the Eastern Ratsnake (*Pantherophis alleghaniensis*).

Methods:

In 2015, our investigation was focused on three sites in southeastern Virginia; False Cape State Park (36.62971818, -75.90019047), Back Bay National Wildlife Refuge (36.68816205, -75.92289671), and the Virginia Zoo (36.8763071, -76.2782861) (permit numbers: FC-RCP-031015, BKB-A Guthrie, 048445). Forty-two free ranging snakes were manually captured and examined by a veterinarian. Snakes were given a transponder subcutaneously for permanent identification (AVID Identification Systems, Inc., Norco, California, USA). Snakes having skin lesions consistent with SFD were sampled through skin biopsies taken using previously described methods (Guthrie et al., 2016). Samples were submitted to the United States Geological Survey - National Wildlife Health Center for fungal culture, histopathologic examination and PCR testing (Bohuski et al., 2015).

Results:

A total of 42 snakes were manually captured and examined (Table 1). Biopsy samples from three snakes were submitted for diagnostic testing based on skin lesions consistent with SFD.

All three of these snakes were positive for SFD on multiple diagnostic tests.

Table 1: Results of snake fungal disease testing in southeastern Virginia in 2015. Back Bay National Wildlife Refuge (BBNWR), False Cape State Park (FCSP), Virginia Zoo (VZ), Brown Watersnake (BWS), Eastern Cottonmouth (CM), Eastern Black Racer (EBR), Northern Watersnake (NWS), Northern Rough Greensnake (NGS), Common Ribbonsnake (CRS), Common Rainbow Snake (CRS), Eastern Gartersnake (EGS), Eastern Rat Snake (BRS), Northern Brownsnake (NBS)

Snake ID	Cap Date	Cap Loc	Species	Sex	Body Wt (g)	Body length (cm)	Lesions (Y/N)	Biopsy (Y/N)	Culture (+/-)	Histo (+/-)	PCR (+/-)
15-001	4-11-15	BBNWR	BWS	F	1860	150	Y	Y	+	+	+
15-002	4-11-15	BBNWR	BWS	F	1520	150	Y	N			
15-003	4-11-15	BBNWR	CM	M		91	N	N			
15-004	4-11-15	BBNWR	CM	M	350	82.5	N	N			
15-005	4-11-15	BBNWR	EBR	M	190	100.5	N	N			
15-006	4-11-15	BBNWR	CM	F	310	74	N	N			
15-007	4-11-15	BBNWR	BWS	F	2070	143	N	N			
15-008	4-11-15	BBNWR	CM	M	410	81	N	N			
15-009	4-11-15	BBNWR	BWS	F	1310	128	Y	Y	+	+	+
15-010	4-11-15	BBNWR	BWS	M	80	86	Y	N			
15-011	4-15-15	VZ	NWS	M	330.1	88	N	N			
15-012*	5-2-15	BBNWR	BWS	F	1840	157	Y	N			
15-013	5-2-15	BBNWR	CM	F		64	N	N			
15-014	5-2-15	BBNWR	NGS	M	40	79	N	N			
15-015	5-2-15	BBNWR	CR	M	30	63	N	N			
15-016	5-2-15	BBNWR	CM	M	850	103	N	N			
15-017	5-2-15	BBNWR	CRS		220	80	Y	Y	-	+	+
15-018	5-2-15	BBNWR	CM	M	720	101	N	N			
15-019	5-5-15	VZ	EGS		73.3	66	N	N			
15-020	5-13-15	VZ	BRS	M	1070	172	N	N			
15-021	6-7-15	BBNWR	CM	F	1070	81	N	N			
15-022	6-7-15	BBNWR	BRS	M	700	117	N	N			
15-023	6-7-15	FCSP	BRS	M	480	135	N	N			
15-024	6-7-15	FCSP	CM	M	340	77	N	N			
15-025	6-7-15	FCSP	NWS	F	240	64	N	N			
15-026	6-7-15	FCSP	CM	F	1020	70	N	N			

15-027	6-7-15	FCSP	NWS	F	230	75	N	N			
15-028	6-7-15	FCSP	BWS	F	950	122	N	N			
15-029	6-7-15	FCSP	NWS	M	120	72	N	N			
15-030	6-7-15	FCSP	BWS		230	81	N	N			
15-031	6-7-15	FCSP	NBS	F		30	N	N			
15-032	6-7-15	FCSP	BWS	F	490	110	N	N			
15-033	6-7-15	FCSP	BWS	M	320	90	N	N			
15-034	6-7-15	FCSP	BWS	F	110	70	N	N			
15-035	6-7-15	VZ	NWS	M	225	81	N	N			
15-036	7-16-15	VZ	BRS	M	45	63	N	N			
15-037	7-18-15	VZ	NGS	M	19	59	N	N			
15-038	8-24-15	VZ	EGS	F	105.4	75	N	N			
15-039	8-28-15	BBNWR	CM	M		77	N	N			
15-040	8-28-15	BBNWR	CM	M	220	68	N	N			
15-041	8-28-15	BBNWR	BWS	F	2090	136	N	N			
15-042	9-18-15	VZ	NGS	F	26		N	N			
15-043	10-9-15	VZ	EGS	M	30	51	N	N			

* same individual as 15-001, snake was captured twice in 2015.

Forty-two individual snakes were captured in 2015; one female Brown Watersnake was captured twice. Snakes captured included 12 Brown Watersnakes (*Nerodia taxispilota*), 12 Eastern Cottonmouths (*Agkistrodon p. piscivorus*), 1 Eastern Black Racer (*Coluber c. constrictor*), 5 Northern Watersnakes (*Nerodia s. sipedon*), 2 Northern Rough Greensnakes (*Opheodrys aestivus*), 1 Common Ribbonsnake (*Thamnophis s. sauritus*), 1 Common Rainbow Snake (*Farancia e. erytrogramma*), 3 Eastern Gartersnakes (*Thamnophis s. sirtalis*), 4 Eastern Ratsnakes (*Pantherophis alleghaniensis*), and one Northern Brownsnake (*Storeria d. dekayi*). Three snakes, two Brown Watersnakes and one Common Rainbow Snake, were confirmed positive for SFD with fungal culture, histopathology and PCR testing.

Discussion:

In 2014, we captured 30 non-venomous snakes and 8 (27%) were SFD positive; in 2015, we captured 42 snakes and 3 (7%) were SFD positive. Interestingly, in 2015 we captured 12 Eastern Cottonmouths (*Agkistrodon p. piscivorus*); none of these animals had skin lesions consistent with SFD. This is notable because some other North American pit viper species such as the Eastern Massasauga and Timber Rattlesnake have suffered significant population declines due to SFD (Allender et al., 2015b; Lorch et al., 2015). Experimental models have inoculated Eastern Cottonmouths with Oo and caused clinical disease (Allender et al., 2015a). There is evidence that disease severity is likely variable between individuals or species (McBride et al.,

2015; Guthrie et al., 2016). Overall, the snakes we examined, including the SFD positive ones, appeared clinically healthy.

Oo acts as a primary pathogen and may be transmitted via direct contact between individuals and/or indirect infection via environmental exposure (Sutherland et al., 2014; Lorch et al., 2015; Rzadkowska et al., 2016). Recommended control measures for preventing the spread of SFD are lacking (Rzadkowska, et al., 2016) but the United States Geological Survey – National Wildlife Health Center recommends wearing clean disposable gloves when handling sick or dead snakes. Supplies and field equipment should be cleaned with soap and water followed by disinfection with a 10% bleach solution. When SFD is known to occur in a region, snakes whose skin lesions appear to resolve with supportive care and/or antifungal therapy may be candidates for release at their capture site. However, these individual should not be released in an area where the disease has not been previously as it is not known if treated snakes may still harbor viable fungus.

A recent study demonstrated that bleach was effective at inactivating Oo using either a 3% or 10% solution at 2-, 5-, and 10-minute contact times. Additionally, some common household cleaners such as Lysol products, CLR, and 409 were effective. However, chlorhexidine, Simple Green, and spectracide were ineffective at killing Oo spores (Rzadkowska et al., 2016). Mud or leaf litter should be removed from equipment and shoes before application of disinfectant to ensure adequate exposure (Rzadkowska et al., 2016).

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