DOI: 10.7589/JWD-D-21-00010

Journal of Wildlife Diseases, 57(4), 2021, pp. 980–982

Investigating the Impact of Group Holding on the Transfer of *Ophidiomyces ophidiicola* DNA in Free-ranging Lake Erie Watersnakes (*Nerodia sipedon insularum*)

Kennymac Durante,[1,4] Ellen Haynes,[1] Kathryn Vivirito,[1] Kristin Stanford,[2] and Matthew C. Allender[1,3] [1]Wildlife Epidemiology Laboratory, Department of Veterinary Clinical Medicine, University of Illinois College of Veterinary Medicine, 2001 S Lincoln Ave., Urbana, Illinois 61802, USA; [2]Franz Theodore Stone Laboratory, The Ohio State University, 878 Bayview Ave., Put-In-Bay, Ohio 43456, USA; [3]Chicago Zoological Society, Brookfield Zoo, 3300 Golf Rd., Brookfield, Illinois 60513, USA; [4]Corresponding author (email: durante4@illinois.edu)

ABSTRACT: Ophidiomycosis threatens snakes worldwide. We swabbed free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) for quantitative PCR detection of *Ophidiomyces ophidiicola* before and after group and individual holding in pillowcases. Our results indicate that group, rather than individual, holding does not significantly increase detection of *O. ophidiicola* DNA.

Ophidiomycosis, caused by *Ophidiomyces ophidiicola* (Allender et al. 2015a), has been reported in numerous snake species and commonly presents as crusts and necrotic scales (Lorch et al. 2016; Baker et al. 2019). Transmission is hypothesized to involve contact between individuals (Lorch et al. 2016; McKenzie et al. 2020). Ophidiomycosis was first reported in Lake Erie watersnakes (LEWS; *Nerodia sipedon insularum*) in 2009 (Lorch et al. 2016) and hundreds of individuals are captured with minimal biosecurity during annual population monitoring. Our objective was to determine the effect of holding methods on *O. ophidiicola* detection in LEWS. We hypothesized that snakes kept in group pillowcases would show increased *O. ophidiicola* DNA quantity compared with snakes kept in individual pillowcases.

Snakes (n=89) were captured by hand at three sites (Gibraltar Island, Kelleys Island State Park, and Middle Bass Island State Park) in Lake Erie, Ohio, US, with hands being covered by clean nitrile gloves or cleaned with alcohol-based hand sanitizer before each capture. Each snake was secured in its own clean transport pillowcase before sampling. Pillowcases were not tested with quantitative (q)PCR but were washed with diluted bleach before each use based on previous disinfection work (Rzadkowska et al. 2017). Snakes were visually inspected by two examiners (K.D. and E.H.) for lesions suggestive of ophidiomycosis (Baker et al. 2019). A full-body swab (S1) was collected from each snake by passing a cotton-tipped applicator along all surfaces of the body. Snakes were then placed in clean group or individual pillowcases in an alternating fashion, for a total of 15 snakes in each site-specific group pillowcase. Snakes remained in these exposure pillowcases for at least 30 min before a second full-body swab (S2) was collected. Swabs were placed in individual, sterile, 2-mL Eppendorf tubes and frozen at –20 C until processing. Snakes were released at their original capture location. All work was approved by The Ohio State University's Institutional Animal Care and Use Committee (protocol no. 2013A00000106-R2).

We extracted DNA from swabs and performed qPCR for *O. ophidiicola* detection (Allender et al. 2015b). The quantity of DNA (measured in nanograms per microliter) and quality (absorbance at 260:280 nm) were measured using spectrophotometry (Nanodrop, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were considered positive if the three replicates had a mean cycle threshold value less than the lowest detected standard dilution. Mean fungal quantities (copies per reaction) were standardized to the total quantity of DNA (copies per nanogram of DNA).

Standardized fungal copy number was assessed for normality using the Shapiro-Wilk test; descriptive statistics were tabulated for snakes in group and individual pillowcases, by

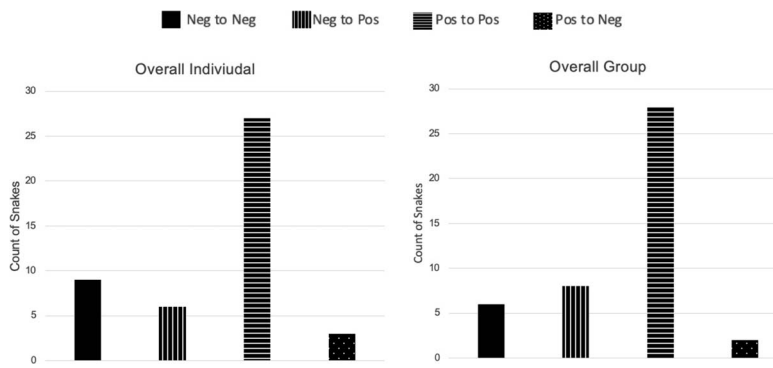

FIGURE 1. Bar graphs showing the number of free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) from three islands in Lake Erie, Ohio, USA, in each of the four categories based on change in quantitative PCR (qPCR) status between initial and postholding swabs of snakes held in either group or individual pillowcases. Neg=negative; Pos=positive.

site and overall. The difference in standardized fungal quantity was calculated between S1 and S2 for each snake. Wilcoxon rank sum tests were performed to compare that difference between animals from group and individual pillowcases within and among sites. To assess whether snakes with a negative S1 were more likely to be positive on S2 if kept in the group pillowcase, each animal was categorized based on S1 to S2 qPCR status: 1) negative to negative, 2) negative to positive, 3) positive to positive, and 4) positive to negative. The prevalence of each category with corresponding 95% confidence intervals (CI) and odds ratios were calculated for animals from individual and group pillowcases at each site and overall. All statistical analyses were conducted using R software, version 3.5.2 (R Development Core Team 2016); statistical significance was assessed at $\alpha=0.05$.

Lesions consistent with ophidiomycosis were detected in 74/89 LEWS. Of the snakes with lesions, 56/74 (76%) were qPCR positive on S1. Overall, 67% of LEWS were initially qPCR positive; 61% were qPCR positive on both S1 and S2 (the Supplementary Table). Of snakes in group pillowcases, 18.2% (95% CI, 8.2–32.7%) converted from negative to positive, whereas 13.3% (95% CI, 5.1–26.1%) of snakes in individual pillowcases also converted from negative to positive (the Supplementary Table and Fig. 1). Snakes that were negative on S1 had no significantly greater odds of testing positive on S2 after being in the group pillowcase compared with those in individual pillowcases. There were no differences in detection among sites.

Our finding that eight initially negative snakes became positive after group holding suggests the possibility of horizontal transfer of *O. ophidiicola*, as recently described by McKenzie et al. (2020). We also documented that six individually housed snakes converted from negative to positive. False-negatives have been reported in the diagnosis of this pathogen but are decreased with thorough swabbing techniques (Hileman et al. 2018), as used in our study. Fungal quantity trended lower on S2, compared with S1 (Fig. 2), with 58% of individuals having a decrease in fungal quantity. This may be due to physical removal of fungus through swabbing or through contact with other snakes or the pillowcase. It is unclear why some snakes from both individual and group pillowcases decreased in fungal quantity between S1 and S2 and others increased.

The high prevalence of qPCR-positive animals decreased this study's ability to evaluate changes in detection of *Ophidiomyces* DNA. Based on the observed proportions of snakes converting from negative to positive in group pillowcases (0.18) compared with individual pillowcases (0.13), the power of our study to detect a statistically significant difference was 0.08. To achieve a power of

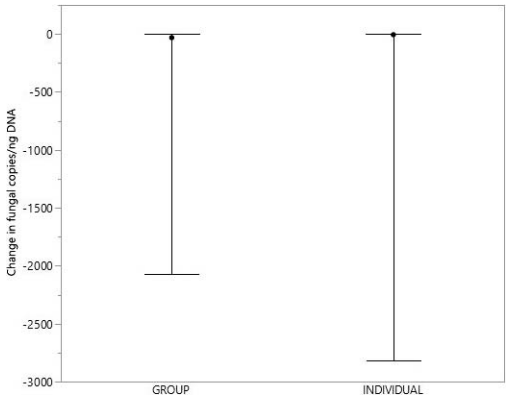

FIGURE 2. Median difference in standardized fungal copies between initial and postholding swabs in free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) from three islands in Lake Erie, Ohio, USA, held in group or individual pillowcases. Error bars show the 25th and 75th percentiles.

0.8 with an α level of 0.05 and our sample size for this study, the difference between the proportions would need to be at least 0.25. Therefore, we recommend repeating this study in a population with a lower pathogen prevalence to achieve a larger effect size or in a similar population with a larger sample size.

Biosecurity is critical when working with wildlife pathogens. Studies have analyzed disinfectant efficacy for specific pathogens (Johnson et al. 2003; Bryan et al. 2009; Rzadkowska et al. 2016), and recent work found that capture method is important in the transmission of amphibian pathogens (Mendez et al. 2008; Gray et al. 2018). Our study found no significant differences with the biosecurity measures implemented but because of its low power and the potential disease transmission ramifications of not using biosecurity, we recommend applying our results to management practices with caution.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-00010.

LITERATURE CITED

Allender MC, Baker S, Wylie D, Loper D, Dreslik MJ, Phillips CA, Maddox C, Driskell EA. 2015a. Development of snake fungal disease after experimental challenge with *Ophidiomyces ophiodiicola* in cottonmouths (*Agkistrodon piscivorous*). *PLoS One* 10: e0140193.

Allender MC, Bunick D, Dzhaman E, Burrus L, Maddox C. 2015b. Development and use of a real-time polymerase chain reaction assay for the detection of *Ophidiomyces ophiodiicola* in snakes. *J Vet Diagn Invest* 27:217–220.

Baker SJ, Haynes E, Gramhofer M, Stanford K, Bailey S, Christman M, Conley K, Frasca Jr S, Ossiboff RJ, Lobato D, et al. 2019. Case definition and diagnostic testing for snake fungal disease. *Herpetol Rev* 50:279–285.

Bryan L, Baldwin C, Gray M, Miller D. 2009. Efficacy of select disinfectants at inactivating Ranavirus. *Dis Aquat Organ* 84:89–94.

Gray MJ, Spatz JA, Carter ED, Yarber CM, Wilkes RP, Miller DL. 2018. Poor biosecurity could lead to disease outbreaks in animal populations. *PLoS One* 13:0193243.

Hileman ET, Allender MC, Bradke DR, Faust LJ, Moore JA, Ravesi MJ, Tetzlaff SJ. 2018. Estimation of *Ophidiomyces* prevalence to evaluate snake fungal disease risk. *J Wildl Manage* 82:173–181.

Johnson M, Berger L, Philips L, Speare R. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Dis Aquat Organ* 57:255–260.

Lorch JM, Knowles S, Lankton JS, Michell K, Edwards JL, Kapfer JM, Staffen RA, Wild ER, Schmidt KZ, Ballmann AE, et al. 2016. Snake fungal disease: an emerging threat to wild snakes. *Philos Trans R Soc Lond B Biol Sci* 371:20150457.

McKenzie CM, Oesterle PT, Stevens B, Shirose L, Mastromonaco GF, Lillie BN, Davy CM, Jardine CM, Nemeth NM. 2020. Ophidiomycosis in red cornsnakes (*Pantherophis guttatus*): Potential roles of brumation and temperature on pathogenesis and transmission. *Vet Pathol* 57:825–837.

Mendez D, Webb R, Berger L, Speare R. 2008. Survival of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on bare hands and gloves: hygiene implications for amphibian handling. *Dis Aquat Organ* 82:97–104.

R Development Core Team. 2016. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. Accessed September 2020.

Rzadkowska M, Allender MC, O'Dell M, Maddox C. 2016. Evaluation of common disinfectants effective against *Ophidiomyces ophiodiicola*, the causative agent of snake fungal disease. *J Wildl Dis* 52:759–762.

Submitted for publication 24 January 2021.
Accepted 10 June 2021.

Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-21-00010: Kennymac Durante, Ellen Haynes, Kathryn Vivirito, Kristin Stanford, Matthew C. Allender. Investigating the impact of group holding on the transfer of *Ophidiomyces ophidiicola* DNA in free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum)*

Supplemental Table S1. Number of snakes and prevalence of change in qPCR results for free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) from individual or group pillowcases overall and at each of three sites in Ohio, USA: Gibraltar Island (GIB), Kelleys Island State Park (KI-SP), and Middle Bass Island State Park (MBI-SP). CI = confidence interval, Neg = negative, Pos = positive

Site	Pillowcase assignment	Neg to Neg	Percent (95% CI)	Neg to Pos	Percent (95% CI)	Pos to Pos	Percent (95% CI)	Pos to Neg	Percent (95% CI)	Total
GIB	Individual	5	33.3 (11.8-61.6)	3	20.0 (4.3-48.1)	5	33.3 (11.8-61.6)	2	13.3 (1.7-40.5)	15
GIB	Group	3	21 (4.7-50.8)	4	28.6 (8.4-58.1)	7	50.0 (23.0-77.0)	0	0 (0-23.2)	14
KI-SP	Individual	1	6.7 (0.2-31.9)	1	6.7 (0.2-31.9)	13	86.7 (59.5-98.3)	0	0 (0-21.8)	15
KI-SP	Group	1	6.7 (0.2-31.9)	1	6.7 (0.2-31.9)	12	80.0 (51.9-95.7)	1	6.7 (0.2-31.9)	15
MBI-SP	Individual	3	20.0 (4.3-48.1)	2	13.3 (1.7-40.5)	9	60.0 (32.3-83.7)	1	6.7 (0.2-31.9)	15
MBI-SP	Group	2	13.3 (1.7-40.5)	3	20.0 (4.3-48.1)	9	60.0 (32.3-83.7)	1	6.7 (0.2-31.9)	15
Overall	Individual	9	20.0 (9.6-34.6)	6	13.3 (5.1-26.8)	27	60.0 (44.3-74.3)	3	6.7 (1.4-18.3)	45
Overall	Group	6	13.6 (5.2-27.4)	8	18.2 (8.2-32.7)	28	63.6 (47.8-77.6)	2	4.5 (0.6-15.5)	44