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NOTE

Detection of amphibian chytrid fungus on waterfowl integument in natural settings

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ABSTRACT: The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), the causal agent of the amphibian disease chytridiomycosis, has spread at an alarming rate since its discovery. *Bd* was initially thought to only infect keratinizing epithelial cells in amphibians, a core component of amphibian skin. However, recent studies have detected *Bd* on the integument of non-amphibian hosts. We conducted a survey of 3 duck species (gadwalls, green-winged teals, and mallards) to determine whether *Bd* DNA could be found on their feet. *Bd* was found on the feet, by quantitative PCR, of individuals from all 3 species (5/11 gadwalls, 4/8 green-winged teals, and 13/21 mallards), though there were no significant differences in zoospore presence or load between species. We conclude that these waterfowl species may act as vector hosts for *Bd*, adding to the growing list of potential waterfowl vectors. Future studies are needed to determine whether *Bd* on waterfowl feet is viable and infectious to amphibian hosts.

KEY WORDS: *Batrachochytrium dendrobatidis* · Ducks · Transmission

INTRODUCTION

The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), the causal agent of the amphibian disease chytridiomycosis, has spread at an alarming rate since its discovery and is now found on all continents on which amphibians exist (Olson et al. 2013). As of 2013, >40% of amphibian species tested were found to be infected with the pathogen (Olson et al. 2013), partly due to the relatively low host specificity of the pathogen (Gervasi et al. 2017). The ability of *Bd* to infect numerous amphibian hosts, paired with its almost ubiquitous presence across the globe, has spurred the need for researchers to answer questions on how the pathogen can spread (Longcore et al. 1999, Olson et al. 2013).

Bd was previously thought to only infect keratinizing epithelial cells in amphibians (Longcore et al. 1999), a core component of amphibian skin. Pathogen-induced lesions included hyperkeratosis and interference with normal osmoregulation, with severe cases leading to death as a result of electrolyte disturbances that cause the heart to stop (Voyles et al. 2009). However, recent work has shown that *Bd* can persist in a number of habitats and on multiple non-amphibian hosts. For example, studies have shown that *Bd* DNA can be detected in water in laboratory settings, independent of hosts, for up to 7 wk (Johnson & Speare 2003, 2005). Additionally, studies have shown that *Bd* can persist in natural water bodies year-round (Hyman & Collins 2012, Chestnut et al. 2014), though the testing sensitivities in those studies could not differentiate between live/infective or dead zoospores. Nonetheless, these combined laboratory and field studies suggest that *Bd* may be able to survive and reproduce in natural water bodies, independent of the presence of potential amphibian hosts.

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The possibility that *Bd* could persist in natural water bodies is concerning as it leads to the possibility of *Bd*-naïve amphibians that may enter habitats with free-living *Bd* zoospores, increasing the possibility of infection(s) to susceptible hosts. Additionally, *Bd* that persists in the environment could also be transmitted via non-amphibian species acting as vectors or reservoirs of the pathogen. McMahon et al. (2013) showed that crayfish can succumb to *Bd*-induced pathology when placed in water that previously contained a *Bd*-infected amphibian host, suggesting that zoospores can remain infective outside of hosts and that crayfish may facilitate in the spread of the pathogen (but see Betancourt-Román et al. 2016, who showed no evidence of *Bd*-induced pathology in crayfish). Another study detected *Bd* on the skin of snakes (Kilburn et al. 2011). Each of these potential alternative hosts, or at the very least vehicles for *Bd* spread, has a relatively limited habitat range. In terms of dispersal ability, waterfowl provide the greatest potential for long-distance spread.

Many waterfowl species migrate hundreds or thousands of miles during migration. In relation to *Bd*, Johnson & Speare (2005) found that *Bd* may persist on the wings of chickens and ducks in laboratory conditions. Garmyn et al. (2012) tested the keratinous toes of 2 geese species for *Bd* and found that 15% tested positive for the fungus. Further laboratory tests, in controlled, sterile conditions, showed *Bd* chemotaxis to, adhesion on, and survival in geese toes, suggesting that waterfowl may serve as a vector for *Bd*. The goal of our survey was to determine whether *Bd* could be found in other waterfowl species.

MATERIALS AND METHODS

Wild ducks (11 gadwalls *Anas strepera*, 8 green-winged teals *Anas carolinesis*, and 21 mallards *Anas platyrhynchos*) were harvested as part of the Arkansas waterfowl hunting season in October of 2012. Immediately after harvesting, the plantar surface and toe webbings of each foot was rigorously swabbed for 60 s using cotton-tipped applicators (Fisherbrand, catalog no. 23-400-116). A different pair of nitrile gloves was used between each subject to prevent contamination. Infection status (*Bd*-positive or -negative) of all experimental animals was confirmed using qPCR. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (catalog no. 69506) from the swabs taken from the feet.

For qPCR analysis, standards were obtained from CSIRO laboratories in Australia and were the same as those used in Boyle et al. (2004). The standards served as the positive controls and each plate contained a negative control (which tested negative on all plates), and all samples were run in triplicate. We used the same 'fast' qPCR technique detailed and proven successful by Kerby et al. (2013). For calculations of prevalence, swabs were categorized as *Bd*-positive when zoospore equivalents were ≥1 (Vredenburg et al. 2010). We used ANOVA to test for an effect of species on *Bd* presence and load using the 'car' package in R, version 3.0.1.

RESULTS

Results of the qPCR analysis showed that 55% of duck feet tested positive for *Bd*. All species tested positive for *Bd*: 45% of gadwalls (5/11), 50% of green-winged teals (4/8), and 62% of mallards (13/21). ANOVA indicated that there were no significance differences in zoospore loads between species ($F_{2,37}$ = 1.200, p = 0.313), nor were there significant differences in *Bd* presence/absence between species ($F_{2,37}$ = 0.892, p = 0.668). *Bd* zoospore loads ranged from 1.167 to 6.899 genomic equivalents (GE) with an average load per swab of 2.344 GE (see Appendix Table A1 for individual loads).

DISCUSSION

Our results suggest that ducks may serve as a vector for *Bd*. As all of the species in the present study tested positive for *Bd* zoospores and there were no significant differences between species, due in part to low sample size and zoospore loads, we cannot conclude that *Bd* shows an adhesion preference for one species of duck over another. However, because we used the protocol of Hyatt et al. (2007), who showed that it is difficult to obtain false positives from said protocol, we are confident in our determination that the ducks tested were *Bd*-positive.

While Garmyn et al. (2012) showed that *Bd* zoospores were able to adhere to waterfowl scales and Burrowes & De la Riva (2017) found *Bd* DNA in preserved aquatic bird museum samples, we must consider that the *Bd* detected in our study could come from other environmental sources, i.e. the water bodies from which the ducks were collected (Hyman & Collins 2012). Kolby et al. (2015) found that *Bd* persisted on leaves where amphibians were previously

situated. Moreover, analyses from Chestnut et al. (2014) showed that *Bd* persists in water bodies year-round and can easily be detected, independent of hosts. Since the ducks from our study were pulled primarily from water bodies, and sampled immediately after harvest, some of the *Bd* detected could come from the environment instead of the feet of the ducks.

While we cannot conclude that the waterfowl in our study serve as a vector for *Bd*, the detection of *Bd* on the feet of the ducks in our study suggests this possibility. An increasing number of studies show that *Bd* can persist or be detected on non-amphibian waterfowl vectors (e.g. Garmyn et al. 2012, Burrowes & De la Riva 2017), as can the salamander chytrid fungus *Batrachochytrium salamandrivorans* (Stegen et al. 2017), raising the possibility of *Bd* being dispersed over long distances. Studies must continue to examine the potential for non-amphibian *Bd* hosts, especially those with the potential for long-distance dispersal such as birds.

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Appendix

Table A1. Individual zoospore loads (measured in genetic equivalents, or GE) found per swab in each waterfowl species

Load	*Bd*	Load	*Bd*
Gadwalls		**Mallards**	
2.00	Y	3.23	Y
0.00	N	0.97	N
0.00	N	1.23	Y
1.40	Y	1.90	Y
1.00	N	2.27	Y
1.17	Y	0.00	N
0.87	N	1.73	Y
2.13	Y	0.00	N
0.90	N	3.40	Y
0.00	N	3.23	Y
1.43	Y	3.10	Y
		6.90	Y
Green-winged teals		0.00	N
0.00	N	2.03	Y
0.60	N	0.00	N
3.87	Y	0.00	N
1.60	Y	0.00	N
0.00	N	2.60	Y
1.73	Y	1.97	Y
1.47	Y	1.00	N
0.00	N	1.17	Y

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