First Report of *Batrachochytrium dendrobatidis* (Chytridiomycota: Rhizophysiales) from American Bullfrog, *Rana catesbeiana* (Anura: Ranidae) from Western Arkansas

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Abstract: A single American bullfrog, *Rana catesbeiana*, collected in May 2023 from Polk County, Arkansas, harbored the skin fungus, *Batrachochytrium dendrobatidis* (*Bd*). However, the host showed no obvious pathological signs of infection. Three larval salamanders and six additional anurans collected from the same general locality were negative for *Bd* as well as other amphibian pathogens, including Ranavirus (specifically FV3) and *Batrachochytrium salamandrivorans* (*Bs*). This is the first time *R. catesbeiana* from Arkansas has been reported with *Bd* as well as being the fourth species of amphibian from the state harboring the fungus.

Introduction

Chytrid fungus causes the disease chytridiomycosis, originally produced by *Batrachochytrium dendrobatidis* (*Bd*). It was initially discovered in 1989 and is an amphibian chytrid that causes a skin infection, with an affinity to occur in anurans (frogs and toads) worldwide (Lips et al. 1999). Unfortunately, the disease has caused a serious decline in, and in some instances, extinction of more than 200 species of amphibians worldwide and poses the greatest threat to biodiversity of any known disease (Stuart 2004; Skerratt et al. 2007; Pounds et al. 2016; Collins 2020).

Arkansas supports a great diversity of 56 species and subspecies of amphibians (Trauth et al. 2004). However, to date, only three (5%) species from the state have been reported to be infected with the fungus. Federally endangered Ozark hellbenders, *Cryptobranchus alleganiensis bishopi* Grobman collected from the Eleven Point River in Randolph County...
were reported to harbor *Bd* (Briggler et al. 2008; Hardman et al. 2020). In another study, *Bd* was found in Blanchard’s cricket frogs, *Acris blanchardi* Harper from the Wapanocca National Wildlife Refuge in Crittenden County (Hanlon et al. 2014). Interestingly, no specimens exhibited clinical signs of a *Bd* infection (e.g., lethargy, sloughing of skin), perhaps indicating that *Bd* in this population may persist without yielding to *Bd*-induced mortality. In a third study, frogs collected from the Bald Knob National Wildlife Refuge (White County) and Felsenthal National Wildlife Refuge (Union County), including postmetamorphic *A. blanchardi* from the former and tadpoles of Coastal Plains leopard frog, *Rana sphenoecephala utricularia* (Cope) from the latter site, harbored *Bd* (Rothermel et al. 2008). Obviously, much more work needs to be done in the state to survey additional populations of amphibians for *Bd* and other amphibian pathogens. Here we attempt to help fill that void with examination of four species of amphibians from Arkansas for amphibian pathogens, specifically *Bd*.

**Methods**

**Host collection and processing**

On 31 July and 1 June 2023, the following 10 amphibians from Polk County were collected by hand and examined for *Bd* as well as for other amphibian pathogens, including Ranavirus (specifically FV3) and *Batrachochytrium salamandrivorans* (*Bsal*): three juvenile Ouachita dusky salamanders, *Desmognathus brimleyorum* Stejneger from Grace Fountain Spring at Big Fork (34°29′07.1232″N, -93°58′05.16″W), and adults of four green frogs, *Rana clamitans* (Latreille), two American green treefrogs, *Dryophytes (=Hyla cinerea) cinereus* (Schneider), and a single juvenile American bullfrog, *Rana catesbeiana* (Shaw) from the Ouachita Mountains Biological Station (OMBS) pond (34°27′43.4484″N, -93°59′54.3264″W). We followed the protocols of Livo (2004) and Standish et al. (2018) by swabbing the ventral and dorsal skin of each amphibian (including the hind-toe webbing of anurans) 15 to 20× with 15.24 cm sterile cotton-tipped applicators (Medline, Northland, Illinois) and preserving the swabs in individual sterile vials containing 95% DNA grade ethanol. Several preventative steps were taken to avoid cross-contamination by keeping individuals separate from the time of collection, using a new pair of gloves to handle each specimen, and swabbing individuals before taking their snout-vent length (SVL) measurements. Following processing, all amphibians were photographed and released unharmed at their site of collection. We follow the use of the genus *Rana* versus *Lithobates* for North American ranid frogs following Yuan et al. (2016).

**Molecular techniques**

To examine swab DNA, ethanol was decanted, and swabs were air-dried. Sample DNA was extracted using 150 μL of the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, Massachusetts) following the manufacturer’s instructions. Extracted DNA was analyzed using a multiplex quantitative PCR (qPCR) targeting *B. dendrobatidis* (*Bd*), *B. salamandrivorans* (*Bsal*) and frog virus 3-like Ranavirus as described by Standish et al. (2018). A synthetic gBlock® containing all three amplicons was used as a positive control and standard curve of 10× dilutions ranging from 10⁷ to one copy per reaction was used to quantify copies per μL (Standish et al. 2018). Sample concentration (copies per ng total DNA) was calculated using a Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific) and the Qubit™ DNA High Sensitivity Kit (Thermo Fisher Scientific) following the manufacturer’s instructions.

**Results**

Ranavirus and *B. salamandrivorans* were not detected. One of 10 (10%) of the amphibians sampled harbored *Bd*. The host was a juvenile *R. catesbeianus* collected from the OMBS pond. The specimen appeared otherwise healthy and exhibited no overt symptoms of the infection. All other amphibians sampled were negative for the presence of pathogens.

No significant inhibition was observed in and qPCR reactions and amplification was only observed in one sample of this host. Samples...
were initially screened (not quantified) then archived at -80°C as we did not recognize the significance of this positive result. After two weeks samples, extracts were quantified and copy number determined. An average Cq value of 30.99 was observed across triplicate reactions which contained an average of 2382 copies of the Bd target amplicon or 9102 Bd copies per ng of total DNA.

**Discussion**

Interestingly, American bullfrogs have been reported to be seemingly resistant (asymptomatic) to the disease and may act as vectors or reservoir hosts (Daszak et al. 2004; Hanselmann et al. 2004). Although the factors are not fully understood, one characteristic that appears to protect R. catesbeiana from Bd involve the production of immunoprotective peptides (Eskew et al. 2018). This resistance has been thought to allow R. catesbeiana to spread Bd to various parts of North America and even other continents (Jenkinson et al. 2016; Yap et al. 2018). In addition, Garner et al. (2006) detected Bd infections in introduced R. catesbeiana from six countries, including Brazil, Canada (British Columbia), France, Italy, United Kingdom, and Uruguay; they also found Bd in introduced American bullfrogs from Arizona, USA. Standish et al. (2018) identified Bd and FV3 from 33.3% and 16.7%, respectively, of adult R. catesbeiana sampled in Wisconsin with none of the bullfrog tadpoles sampled testing positive for either pathogen. Rothermel et al. (2008) reported five of 229 (2%) individuals of R. catesbeiana from Georgia, North Carolina, and Virginia harbored Bd but specimens from Florida, Louisiana, Mississippi, and South Carolina were not infected; no R. catesbeiana from Arkansas were sampled. However, in adjacent Oklahoma, several studies have reported Bd in R. catesbeiana from the state either rarely or in high prevalence (Watters et al. 2016, 2018, 2019, 2021; Marhanka et al. 2017; Nichols et al. 2022).

We suggest additional amphibians from the state be surveyed for Bd as well as other amphibian pathogens. Undoubtedly, more individuals will be discovered to harbor this fungus, including the possibility, more importantly, of finding it in Arkansas endemic and protected species.

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**References**


Bd in Rana catesbeiana


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