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# SUCCESSFUL TREATMENT OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN EASTERN HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) WITH TERBINAFINE

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**Abstract:** In 2019, two wild-caught adult female eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) received a preshipment examination and were individually swabbed for chytrid testing via quantitative polymerase chain reaction (qPCR). Physical examination was unremarkable. Both females tested positive for *Batrachochytrium dendrobatidis* (*Bd*) and negative for *B. salamandrivorans* (*Bsal*). A course of terbinafine hydrochloride 1% in alcohol was administered in a 0.005% treatment bath for 5 min once daily for 5 d. Both animals were individually retested 1, 3, and 4 wk after treatment using qPCR. All post-treatment samples were negative for *Bd* and *Bsal*. This report represents the first successful treatment with terbinafine hydrochloride 1% in alcohol to eliminate subclinical *Bd* infection in eastern hellbenders and underlines the importance of preshipment testing for chytrid in all amphibians being transferred to new facilities or released into the wild as a means to minimize risk of disease introduction via subclinically infected individuals.

## CLINICAL BRIEF

*Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) are two highly infectious fungal agents responsible for chytridiomycosis.<sup>11,16</sup> *Bd* chytridiomycosis is a potentially fatal disease that has been reported as threatening 39% of the rapidly declining amphibian species.<sup>16</sup> Because of global impact of chytridiomycosis on amphibian populations, the World Organization for Animal Health (OIE) has designated infection with *Bd* and *Bsal* as internationally notifiable diseases.<sup>13</sup>

Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are aquatic salamanders native to the eastern United States and susceptible to infection with *Bd*.<sup>3</sup> A previous study demonstrated skin swabs collected from wild populations of eastern hellbenders in New York, Pennsylvania, Ohio, and Virginia tested positive for *Bd* using duplex quantitative polymerase chain reaction (qPCR).<sup>3</sup> All positive samples had low loads of *Bd* zoospores compared with swabs collected from other *Bd*-susceptible species, suggesting eastern hellbenders can be capable asymptomatic carriers.<sup>3</sup> However, hellbenders infected with *Bd* in human care may succumb to disease.<sup>9</sup>

In 2014, two young adult female eastern hellbenders (case 1 and case 2) were acquired by

The Wilds as part of a headstart and education program. Blood was collected for a complete blood count (CBC) and serum biochemistry. Skin swabs were collected for *Bd* and *Bsal* testing using standard anuran protocol of gently swabbing the ventral surfaces of the skin approximately 20 to 30 times.<sup>15</sup> Five passes of a fine-tip swab on the target areas of the pelvic patch, ventral thighs, and toes of each foot were completed, and animals were individually housed for a 30-d quarantine period. Serum biochemistry revealed markedly elevated creatine phosphokinase (CPK) in both individuals, whereas remaining analytes were within normal limits. CBC samples were lost by the laboratory. *Bd* testing was positive for case 1 and negative for case 2. Case 1 was treated by heating the tank water to 90°F for 48 h according to standard quarantine protocols at that time. Following heat treatment, skin swabs tested negative for *Bd* by qPCR.

Following a 3-day acclimation period, a quarantine examination was performed. Physical examination revealed varying degrees of *Saprolegnia* infection in both cases 1 and 2, but the remainder of examination was unremarkable. Unfortunately, no further description of lesions was recorded in the medical notes.

Treatment consisted of methylene blue baths q24h until the *Saprolegnia* infection was visibly resolved according to standard quarantine protocols at that time. Saprolegniasis and elevated CPK levels were attributed to recent handling and transport. Saprolegniasis and *Bd* were considered cleared at the end of quarantine based on physical examination and qPCR, respectively, and no

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further treatment was warranted. Following quarantine, cases 1 and 2 were housed together in a 500-gallon stream tank with a mesh divider between the two individuals. Life support consisted of a sand filtration system and weekly testing for nitrates, nitrites, ammonia, and pH. Diet consisted of frozen-thawed pinkie mice and smelt every other day.

In 2019, case 1 was examined, and skin swabs were collected using standard anuran protocol, as part of a preshipment protocol. Samples were tested for *Bd* and *Bsal* using qPCR. Physical examination was unremarkable, and water quality was within normal limits.

Skin swabs for case 1 were qPCR positive for *Bd* and negative for *Bsal*. Repeated sampling and qPCR were performed to confirm the results. Because of sharing the same water system as case 1, case 2 was tested for *Batrachochytrium* spp via the same methods described. Case 2 was qPCR positive for *Bd* and qPCR negative for *Bsal*. Tank water was filtered with a 0.45- $\mu$ m cellulose nitrate filter, extracted using a published modification of the Qiagen DNeasy kit protocol for filter samples, and tested for *Batrachochytrium* spp. via qPCR, and results were negative for *Bd* and *Bsal*.<sup>8</sup> Cases 1 and 2 were briefly quarantined in separate 55-gallon tanks but this led to signs of stress, notably skin secretions, after removing the individuals from their shared tank. Because of stress, in addition to the negative water sample, both hellbenders were moved back into their original tank, and a 50% water change was performed.

Water heating was used in 2014 to treat *Bd* as part of the quarantine protocol. However, water heating can be a stressful experience for aquatic salamanders because of low tolerance to temperatures outside of their natural environmental conditions.<sup>7</sup> To limit unnecessary stress and simplify treatment, terbinafine hydrochloride (HCL) solution 1% in alcohol (LamisilAT Spray, GSK Group of Companies, Brentford, Middlesex TW8 9GS, United Kingdom) was chosen for treatment, based on successful results in amphibians in a previous study.<sup>6</sup> Treatment consisted of combining 15 ml terbinafine HCL 1% in alcohol with 3 L tank water in a 10-gallon tank creating a 0.005% bath. Using separate treatment tanks, each hellbender was soaked for 5 min q24h for 5 d.<sup>6</sup> Cases 1 and 2 were returned to their original tank system between treatments throughout the duration of treatment.

Cases 1 and 2 remained active and exhibited normal behavior and appetite during and after treatment. Skin swabs were collected individually

from cases 1 and 2 at 1, 3, and 4 wk after treatment for qPCR testing for *Batrachochytrium* spp as previously described. All swabs were qPCR negative for *Bd* and *Bsal*. Both cases were deemed free of *Bd* infection based on multiple negative qPCR results 4 wk after treatment.

Chytrid testing was performed at The Wilds Conservation Science Training Center (Cumberland, OH 43732, USA). DNA was extracted from swabs using the Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Germantown, MD 20874, USA). Testing for *Batrachochytrium* spp. was performed in triplicate using qPCR in a multiplex using previously published primers and probes and a gblock for a standard curve.<sup>17</sup> All reactions were run on an ABI 7500 real-time PCR system (Thermo Fisher Scientific, Waltham, MA 02451, USA).

Various chemical treatment protocols have been explored in animals PCR positive for *Bd* infection. Continuous chloramphenicol baths at 200 mg/L decreased *Bd* burden and eliminated clinical signs caused by chytridiomycosis without toxicity in hellbenders under human care.<sup>1</sup> Itraconazole is commonly used to treat chytridiomycosis caused by *Bd* in captive animals, but side effects, such as skin irritation and decreased osmotic function, as well as toxicosis, have been reported in various amphibian species, including *Ambystoma dumerilii*, *Ambystoma andersoni*, *Alytes obstetrican*, *Litoria caerulea*, *Incilius nebulifer*, and *Limnodynastes peronii* treated with higher doses.<sup>4,5,12,18</sup> Terbinafine has also been successfully used to clear *Bd* infection in bullfrogs (*Lithobates catesbeianus*) and six other amphibian species using LamisilAT Spray at 0.005% and 0.01% and a generic terbinafine dissolved in alcohol at 0.005%, respectively.<sup>6</sup>

Based on the low cost, simple treatment protocol, and short duration of treatment, terbinafine was chosen as the treatment option for these cases. Although terbinafine HCl at 0.01% was reported to not be universally effective for treatment of experimentally infected alpine tree frogs (*Litoria verreauxii alpine*) or naturally infected common eastern froglets (*Crinia signifera*), a study proposed that terbinafine HCl at 0.005% can successfully treat *Bd* infection in naturally infected species that tend to have low levels of zoospores, which is supported by the current clinical brief.<sup>14</sup> Because of species variation, treatment protocols for *Bd* should be tested in each species and life stage to ensure safety and efficacy.<sup>2</sup>

The source of the *Bd* reinfection in cases 1 and 2 was not determined. *Bd* occurs in the environment,<sup>10</sup> so despite biosecurity protocols including limited building access, daily preparation of a bleach foot bath used for entry, dedicated tank equipment, and frequent hand washing, it is possible that *Bd* from the environment was able to contaminate the hellbender enclosure. In addition, *Bd* copy number was between 7 and 22 copies/ $\mu$ l for cases 1 and 2, so *Bd* may have been present in the tank water but below detection thresholds. However, according to the program protocol used since the commencement of the headstart program, individuals are qPCR tested for *Bd* and *Bsal* before release, and no prior cases of *Bd*-positive hellbenders have been found in previous years. The water source has remained the same since the headstart programs inception and comes from water tanks filled by a local water hauling company that is certified and licensed by the local health department. A separate, more intensive testing study would be needed to identify the source of reinfection.

A limitation of this report includes the swabbing protocol used, which is anuran focused and targets the ventral skin only. Thus, developing a standardized swabbing protocol for aquatic salamanders may be beneficial.

Treatment of *Bd* infection with terbinafine HCl in eastern hellbenders has not been previously reported. This clinical brief documents successful treatment eliminating *Bd* infection in two adult eastern hellbenders, adding to a limited number of therapeutic studies in aquatic salamanders. This report also underlines the importance of routine testing for *Bd* in amphibians before transfer to different facilities or return to the wild. Such testing minimizes risk of disease introduction via a carrier species into a new collection and may improve release success for hellbender headstart programs.

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