First documented case of Ranavirus in New Jersey amphibians

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Abstract

While habitat loss and pollution continue to be significant threats to amphibians, emerging infectious diseases are playing a considerable role in the disappearance of these ecologically important organisms. This study has the long-term objective of using molecular techniques to document and assess the prevalence of amphibian diseases throughout the state of New Jersey. In 2009, we documented the first known occurrence of the chytrid fungus, *Batrachochytrium dendrobatidis*, in the state and now, in 2011, we report the first known occurrence of Ranavirus in New Jersey amphibians. Using a combination of traditional PCR and RT-PCR we show the presence of this emerging infectious disease in both Green Frog tadpoles and Fowler's tadpoles. So far, only tadpoles seem to be affected in this area, with dramatic symptoms being exhibited especially by Green Frog tadpoles. The affected site is being managed for endangered pine snakes and is home to a number of different reptiles and amphibians, including the threatened Pine Barrens treefrog. The presence of such sensitive herpetofauna in an area with a known disease is disturbing. We aim to continue monitoring the site to track the progress of the disease.

Fig. 1. RPD site with numerous dead and bloated Green Frog tadpoles on 05.17.11. Many showed red lesions on their ventral sides

Fig. 2a. Close up of dead Green Frog tadpole, 05.17.11

Fig. 2b. Fowler's toad tadpoles eating the corpse of a dead Green Frog tadpole. 05.17.11

Fig. 2c. Dead and dying Fowler's Toad tadpoles, 05.26.11

Fig. 2a

Fig. 2b

Fig. 2c

Site Description

Animals were sampled from five ponds located in Ocean County, NJ, within an area that has been managed for the benefit of pine snake (*Pituophis melanoleucus*) populations. The first site (Retention Pond D, RPD) is immediately adjacent to a capped landfill. One side of the basin is lined and retains water year-round, while the rest is unlined, and the water level rises and falls with the water table. There is very little vegetation present, and only a minimal amount of algae or other plant material in the ponds. The second and third sites are located in close proximity to one another at the edge of a field that was artificially cleared for pine snake management (Management Field 2). Management Field 2 (MF2) Breeding Pond (BP) is a small artificially constructed, unlined pond. MF2 Vernal Pool (VP) is a small temporary pool. The fourth site (Hay Road Pond, HRP) is a large, heavily vegetated permanent pond. The final site (Irrigation Pond, IP) is a lined irrigation pond at the edge of the property near the roadside, across the road from a major shopping center.

Work Cited

Mao J; RP Hedrick; VG Chinchar. 1997. Molecular Characterization, Sequence Analysis, and Taxonomic Position of Newly Isolated Fish Iridoviruses. *Virology* 229:212-220

Johnson AJ; AP Pessier; JFX Wellehan; A Childress; TM Norton; NL Stedman; DC Bloom; W Belzer; VR Titus; R Wagner; JW Brooks; J Spratt; ER Jacobson. 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. *Journal of Wildlife Diseases* 44:851-863

Materials and Methods

We visited the five ponds on 05.17.11 and 05.26.11. In order to test for the presence of Ranavirus, we took tail clips of those Green Frog (*Lithobates clamitans*) tadpoles (GFTs) that exhibited symptoms but were still alive (Fig. 2a), but took the entire tadpole when the animal was dead (Fig 2b.). Since no Fowler's toad (*Anaxyrus fowleri*) tadpoles (FTs) were exhibiting symptoms on our first trip, we allowed them to swim in approximately 500 µl of pond water (in an Eppendorf tube); we then returned the tadpole and most of the water to the pond, retaining ca. 100 µl on which to perform a DNA extraction. On our second trip, FTs were exhibiting sluggish behavior and many were dying, so we took whole tadpoles for tissue samples.

Analysis: We performed genomic DNA extractions using the QIAmp DNA Mini Kit (Qiagen) on tail clips from GFTs, tail clips or whole tadpoles from FTs (depending on the size of the tadpole), and on the water from tubes in which FTs had been allowed to swim. Traditional PCR was performed on all samples using 2 □l of extracted DNA in a 25 µl reaction containing the following components: 20mM MCP4 and MCP5 primers (Mao *et al.* 1997), 1.5 mM $MgCl_2$, 1X *Taq* buffer, 0.2 mM of each dNTP, and 0.1 U/ml *Taq*, and using the following parameters: 94° C 2.5 min, 25 cycles of 94° C 30 sec, 50° C 30sec, 72° C 30 sec, final extension of 72° C for 10 min (Johnson *et al.* 2008). RT-PCR was performed on the FT swimming water to test for low levels of infection using 11.5 µl of extracted DNA and 2.5 µl of positive DNA. DNA from a GFT that consistently yielded positive results using traditional PCR was used as the RT-PCR positive. The parameters used were as follows: 95º C 10 min, 40 cycles of 95º C 45 sec, 50º C 30 sec, 72º 30 sec.

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3a-c. Various collection methods. a. Tail clip of a GFT that is exhibiting Ranavirus symptoms but is still alive. b. Whole GFT tadpole, dead. c. FT allowed to swim in Eppendorf tube and then returned to water, with residual tube water collected

Acknowledgements

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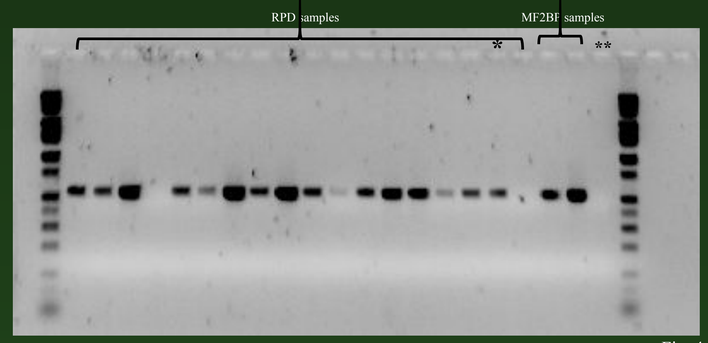

Fig. 4a

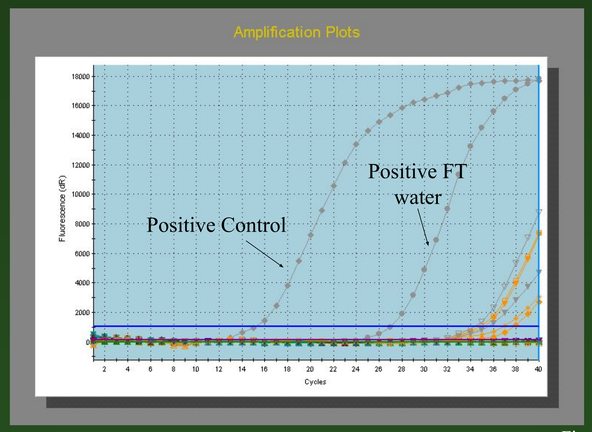

Fig. 4b

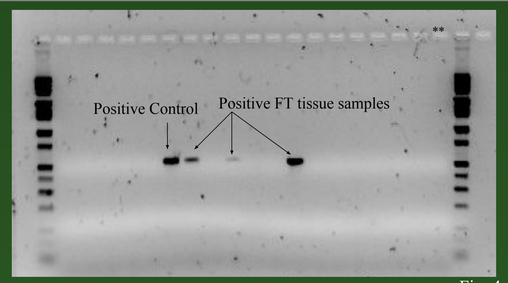

Fig. 4c

Results and Discussion

Fig. 4a-c. Subset of positive Ranavirus results

a. GFTs from RPD and MF2BP showed the presence of Ranavirus. * = GFT corpse on which FT were feeding, ** = Negative Control. Out of a total of 40 samples run from 05.17.11, 19 were positive. No positive samples were found at MF2VP, HRP, or IP.

b. RT-PCR results on FT swimming water from RPD, collected on 05.17.11.

c. FT tissue samples from RPD, collected on 05.26.11. Out of a total of 12 samples run, 3 were positive. ** = Negative Control.

We chose six of the positive GFT samples and sequenced the PCR product after purification using the QIAquick PCR Purification kit (Qiagen). We then performed a BLAST search in GenBank which resulted in all six samples matching Frog Virus 3.