

Distribution of the Iridovirus in Free-Ranging Eastern Box Turtles at Brookhaven National Laboratory.



DANE BUENTEN¹ and VALORIE TITUS^{2,3}

¹ SUNY Fredonia, Fredonia, NY 14063

² Brookhaven National Lab, Upton, NY 11973

³ Binghamton University, Binghamton NY, 13903



The Iridovirus is a family of pathogenic disease that affects reptiles, fish, and amphibians worldwide. Currently, there are four documented genera: The Chloriridovirus and Iridovirus (which infects invertebrates) and the Lymphocystivirus and Ranavirus (which infects cold-blooded vertebrates). A recent outbreak of this Ranavirus had been detected on Brookhaven National Lab (BNL) property among the Eastern Box Turtle population. The symptoms of the Ranavirus in Eastern Box Turtles include conjunctivitis, lethargy, oral and nasal discharge, aural abscesses, and death. Since New York State considers this species a Special Concern, a survey was needed to track and record the size and effect of this outbreak. Oral and cloacal samples from Eastern Box Turtles were collected on site for DNA. These DNA samples were removed from the swabs using the DNeasy tissue kit procedure. The samples were run through Polymerase Chain Reaction twice to enhance the DNA quantity, and then placed into an electrophoresis gel. Using Geographic Information Systems (GIS), the location of all turtles found was plotted so the distribution of the virus could be seen across BNL. The results came back inconclusive for this experiment. The majority of the PCR testing came back negative, however there was reasonable doubt as to whether the outcome was reliable. Also, there was not enough consistency in the GIS plotting to be reasonably sure of the Ranavirus movement.

Introduction

Eastern Box Turtles

- Scientific name – *Terrapene carolina carolina*
- Possesses “boxy” shell and plastron hinge
- Omnivore
- Mainly terrestrial

Iridovirus

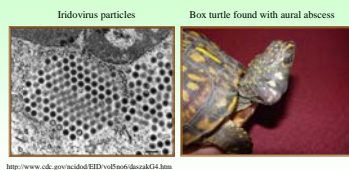
- Icosahedral symmetry
- Diameters of 125 to 300 nm
- Double-stranded DNA 120 to 300 kilobps

Ranavirus

- Member of the Iridovirus family
- Infects fish, amphibians and reptiles
- Observed to be in Eastern Box Turtles at BNL

Iridovirus in box turtles

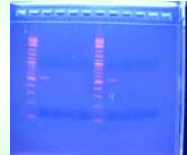
- Multiple observations of iridovirus infections in Eastern box turtles [1,2]
- Two *T. c. Carolinas* were found at Brookhaven National Laboratory in August 2005 with ocular discharge and swelling, aural abscesses, and yellow caseous plaques
- One was found at BNL in August 2007 with advanced symptoms
- Histopathology, PCR, and virus isolation confirmed a ranavirus infection [1]
- Species is listed as Special Concern in New York



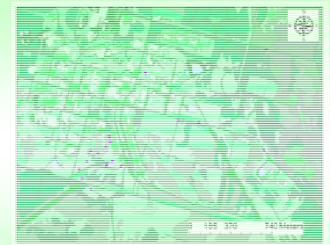
<http://www.cbl.gov/ncid/EBD/vol06/dec04/04.htm>

Results

Iridovirus Testing PCR Products Resolved on Agarose Gel



Lane 2 & 7 – Ladder Lane 3 & 8 – Prior Positives
Lane 4-6 & 9-11 – Tissue samples



GIS points of collected Box Turtle samples



Oral samples being collect near Grover St.



Different angles of an Iridovirus infected box turtle

Materials and Methods

- Oral and cloacal tissue samples were collected from turtles located on BNL property. Box Turtles were located using systematic transect searching and chance encounter.



- Turtles were labeled with a scute notching identification system. There mass, plastron width, plastron length, carapace length, and local temperature reading were taken.



- DNA tissue samples were extracted using the Dneasy© kit (Quiagen®). Extracted DNA was stored at -20° C for prolonged storage.



- Using the sense (5'-GACTTGGCCACTTATCAC-3') and anti-sense (5'-GTCTCTGGAGAAGAAGAA-3') primer, DNA could be amplified. Materials and protocols were given by New England Biolabs' Taq PCR Kit, which contained primer, distilled water, standard reaction buffer, dNTP, Mg²⁺, and Taq. DNA was run through the thermal cycler twice. PCR products were placed in a 0.2% agarose gel for electrophoresis and examination.



Discussion and Conclusion

- At this time no iridovirus infections in 2008 samples have been identified.
- There is still a high probability that the Iridovirus is still located on BNL property. Past studies have found infected turtles as early as last year. [4]
- Virus could have migrated to a different location at BNL, as Weaver Pond has been the focus of most turtle observations. Earlier findings have found infected turtles at different locations.
- Turtles have been observed to have a home range of 1.756 - 8.175 ha. Although fairly small, this range tends to overlap with many other turtles. This may be ideal for iridovirus transmission. [5]
- It has been suggested that PCR testing could be insufficient in detecting pre-symptomatic turtles. [4]
- In order to confirm the validity of PCR, other techniques should be utilized such as histopathology.
- However, samples of dead turtles are needed, as histopathology requires tissue samples
- Two Eastern Box Turtles were found with excess oral discharge near Weaver pond in 2008. Both tested negative for the Iridovirus.
- Turtles were treated and found healthy.
- Further research can include:
 - More surveys on turtle sampling and Iridovirus detection.
 - Discovering methods to treat infected turtles of Iridovirus symptoms.
 - Understanding the oral discharge found on two Box turtles.

Acknowledgements

I would like to thank the Department of Energy, Brookhaven National Laboratory, and the Office of Education Programs for the opportunity to participate in this extraordinary SULI program. I would like to thank Tim Green for his sage advice in all matters. I would like to express my deepest gratitude to my mentor Valerie Titus for all the guidance and support she has given me. I would also like to thank my colleagues Carmen Maldonado, Tyra Bunch, Sarah Miloski, and Stephanie Zivogel for all their help both in and out of the field. It has been an honor to work with such peers.

References

- [1] Johnson et al. (in review) Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States.
- [2] De Vos, R. et al. (2004) Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). *Journal of Zoo and Wildlife Medicine*, 35: 534-543.
- [3] Dodd, C. K. Jr. (2001) North American Box Turtles: A Natural History. University of Oklahoma Press, Norman.
- [4] Snyder, S., Titus, V. "Spatial distribution of Iridovirus in the Eastern box turtle population at Brookhaven National Laboratory: Implications for transmittance based on home range size." Unpublished
- [5] Rickard, L. F. (1989) Home Range Behavior Among Box Turtles (*Terrapene c. Carolina*) of a Bottomland Forest in Maryland. *Journal of Herpetology*, 23: 40-44.