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

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Abstract

Distribution of the Iridovirus in Eastern Box Turtles at Brookhaven National Lab. DANE BUENTEN (SUNY Fredonia, Fredonia, NY 14063) VALORIE TITUS(Brookhaven National Lab, Upton, NY 11973)

The Iridovirus is a family of pathogenic disease that affects reptiles, fish, and amphibians worldwide. Currently, there are four documented genera: The Chlorirdovirus 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, 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. A group of oral and cloacal samples of 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 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.

I. Introduction

Terrapene carolina carolina, also known as the Eastern Box Turtle, is a species of turtle found in New York State. Its name is composed of two parts; *Terrapene* is derived from the Algonkian Indian name for "little turtle", while *Carolina* comes from where it was first described [1]. *T. c. carolina* are characterized by their boxy carapace like appearance, as well as their ability to completely retreat into their shell using a plastron "hinge". Eastern Box Turtles grow to be an average carapace length of 10.0-16.0 cm and an average plastron width of 6.0-10.0 cm [1]. The males typically have red eyes, brighter skin patterns, and a concave plastron utilized in mating. The females typically have brown eyes and a level plastron. Eastern Box Turtles are omnivores; their diet consists of berries, roots, leaves, grubs, worms, and carrion [1]. It is suggested that turtles will be more carnivorous when they are younger, while they become more herbivorous as they age. [1]

The Iridovirus is a family of pathogenic viruses that affect both vertebrates and invertebrates. It is a DNA-based, icosahedral symmetric virus contained in a lipid membrane that has approximately 120 –300 kilobps [2]. The Chlorirdovirus and Iridovirus (which infects invertebrates) and the Lymphocystivirus and Ranavirus (which infects cold-blooded vertebrates) are part of this family. The Ranavirus is of special interest because it is the only genus that infects fish, amphibians and reptiles. The Ranavirus' name is attributed to its original discovery in the *Rana* genus [3]. The symptoms of this virus in *T. c. carolina* can be lethargy, oral or nasal discharge, conjunctivitis, anorexia, internal lesions, and death.

Little is known about the Ranavirus, other than found in increasing number of reptiles, fish, and amphibians. Whether this is due to an increase of infection, or scientists' improved ability to detect it in recent times is still debatable. It is thought to be a suspect in the recent worldwide decline of amphibian populations [3]. The way it is transferred has not been confirmed, although some evidence suggest that it may be ingested, [3] and can cross species barriers [4, 5]. There is also no effective way to treat an Iridovirus infection. It is found in a relatively small number when contrasted to fish or amphibians, however the most known occurrences of infection in reptiles are found in chelonians [6,7].

In August 2005, two Eastern Box turtles at Brookhaven National Lab tested positive for the Ranavirus [8]. One of these turtles died within 24 hours while the other was sent for treatment. However, this turtle continued to decline until its death in September. Another turtle was found moribund in the same area as the prior two in July 2006 and died shortly after treatment began. PCR tests on liver tissue also confirmed the presence of the virus. On July 2008, two turtles were found with oral discharge. One of the turtles contained so much discharge that its jaws had to be pried open for an oral sample. Although they showed no signs of lethargy or other symptoms, these turtles were of special concern to this research.

II. Materials and Methods

Turtles were found using systematic transect searching on Brookhaven property. Both an oral and cloacal swab was taken from each turtles using cotton swabs. The turtle's carapace, plastron length, plastron width, and mass were measured, as well as the

activities of the turtle at the time of capture. Local weather readings were taken using a Kestrel 3000 weathermeter. The turtles were also systematic notched using a common shell identification system. The DNA on the swabs were extracted using the Quaigen's DNeasy tissue extraction kit procedures and protocols. The extracted DNA was stored in -20°C temperatures to ensure longevity.

Using the sense (5'-GACTTGGCCACTTATCAC -3') and anti-sense (5'-GTCTCTGGAGAAGAAGAA-3') primer, DNA could be amplified. New England Biolabs' Taq PCR Kit, which contained primer, distilled water, standard reaction buffer, dNTP, Mg^{+}, and Taq, was utilized for protocols of PCR. DNA was run through the thermal cycler twice for enhanced amplification.. Once finished, 5 μl of DNA was combined with 5 μl of 2x dye and 10 μl of distilled water. This mixture was loaded into a 2% Agrose electrophoresis gel along with a ladder for reference. The gel was allowed to run for 30 minutes. The gel was then observed under ultraviolet light for DNA bands at 300 base pairs.

III. Results

A total of 19 Eastern Box Turtles and 39 tissue swabs were collected for this study. Laboratory testing came back negative for the Iridovirus in all turtles swabbed. The first turtle that was discovered with oral discharge was sent to the Save the Animals Rescue (STAR) Foundation for treatment. It was deemed healthy and was released back into the wild a week after being caught. The second turtle was also deemed healthy and was released two day after capture. Swabs from previous years are still being analyzed.

IV. Discussion and Conclusion

Turtles captured in the summer of 2008 tested negative for the presence of iridovirus. However, it cannot be assumed that the iridovirus is not present on BNL property until further surveys have been conducted. Infected turtle occurrences have been reported as recently as last year [9]. There might be several reason why the Iridovirus may be present, but not detected in the *T. c. carolina* population. Laboratory testing seemed not to be very consistent throughout this research. One test run of a previously acknowledged infected tissue sample did not come back as positive. This showed that perhaps some of the samples could have had the iridovirus and did not become evident due to a failure in our testing. Another explanation is that the iridovirus is slowly migrating to a different parts of BNL property. Only certain areas of BNL property were surveyed, mostly centered on Weaver pond. However this seems to be somewhat unlikely as pervious research has shown that local Eastern Box Turtles keep to a home range of about 1.756 - 8.175 ha (10). Finally, it has been suggested that in pre-symptomatic turtles, the PCR that is used is not sensitive enough to detect the iridovirus [9]. There might be such trace amounts of virus DNA in the turtle's body before the virus begins to reproduce that it cannot be seen in electrophoresis.

There is also the oral discharge of the allegedly sick turtles to consider. It was believed early on that the two Box Turtles did not have the iridovirus due to the lack of other symptoms, and the laboratory testing supported this belief. If this was not the iridovirus, then what caused it? Was it something in their diet, or was it an illness that little is known about? It was the former, then an exploration on whether the food item is

harmful or merely benign. More investigation into the matter should be done in order to understand the scope of this oral discharge affliction.

IV. Literature Cited

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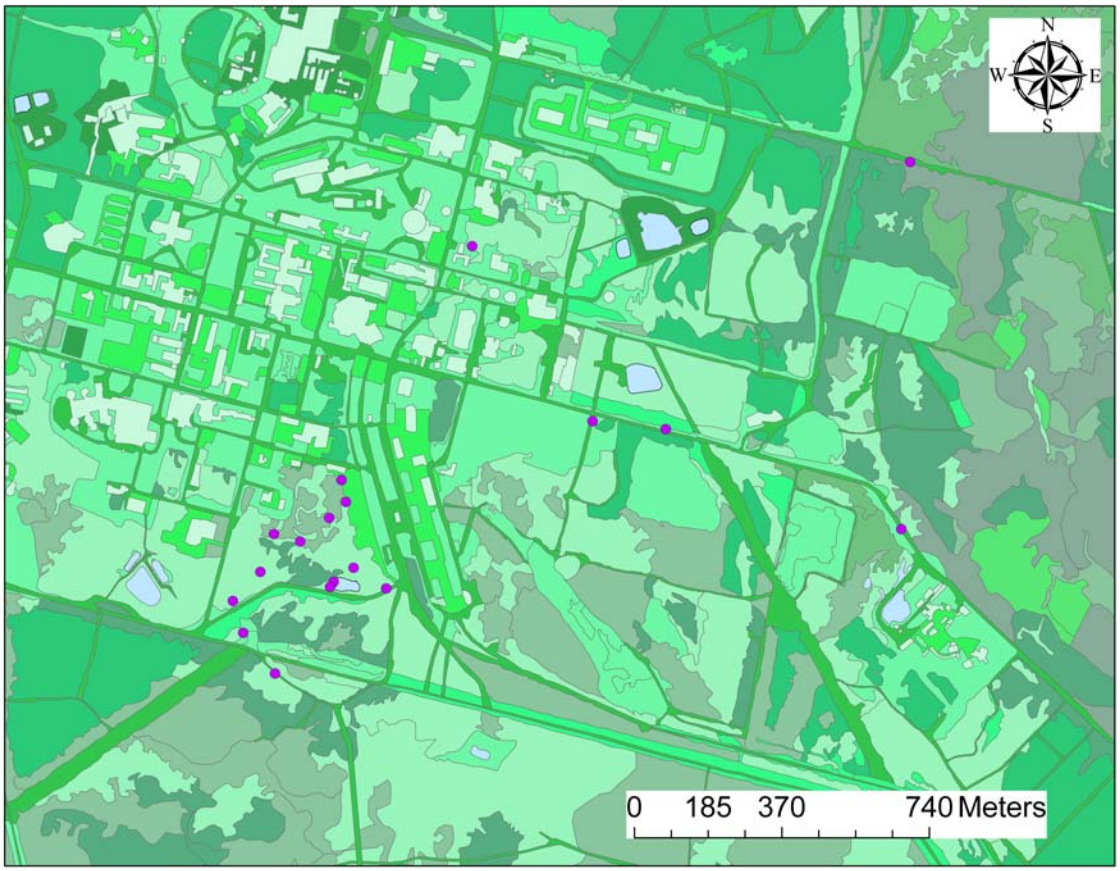
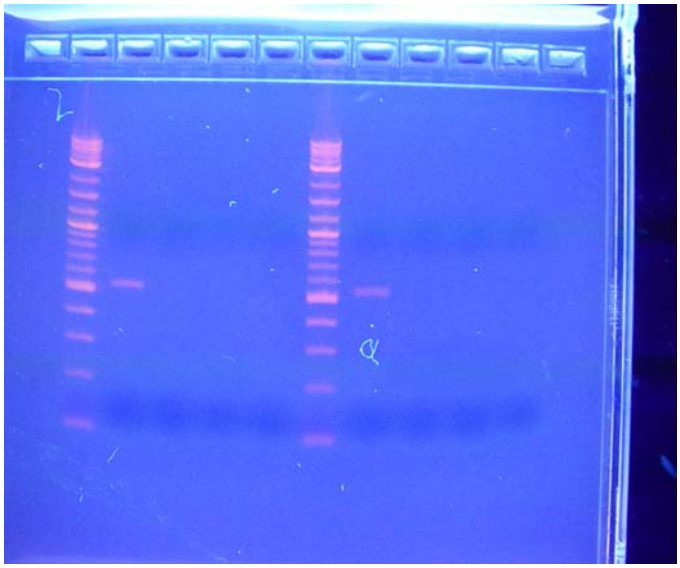
Figures



Plotted points of all Eastern Box Turtles found on BNL property.



Electrophoesis gel of allegedly sick turtles DNA.