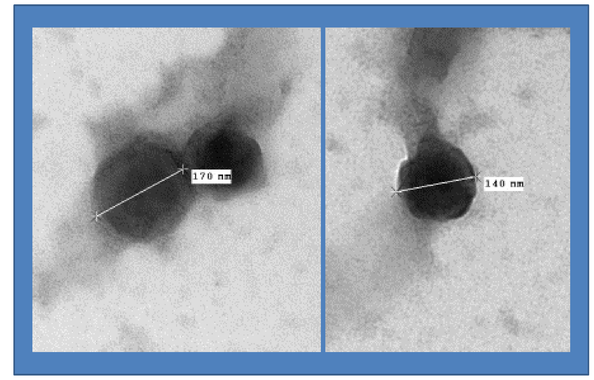
ISOLATION AND PARTIAL SEQUENCING OF A FV-3-LIKE RANAVIRUS FROM THE CARCASS OF A JUVENILE EASTERN PAINTED TURTLE (*Chrysemys picta picta)*

L. Iwanowicz, C. Densmore, C. Hahn

U.S. Geological Survey, Leetown Science Center

Kearneysville, WV. USA



Introduction

In the Spring of 2006, a painted turtle carcass recovered in Kearneysville, WV was presented to the Leetown Science Center. Due to moderate decomposition, a limited necropsy was performed with no remarkable gross findings. Cell-free extract of an internal organ homogenate was inoculated onto BF-2 and EPC cell culture monolayers, producing cytopathic effect that remained evident following serial passage.

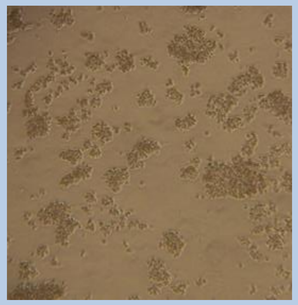


Table 1

Primer Pair	Primer Sequence	Target	Expected Product size (bp)	Amplification*
MCP-F MCP-R	GACTTGGCCACTTATGAC GTCTCTGGAGAAGAAGAA	Major Capsid Protein	530	Yes
DNApolF DNAPolR	GTGTAYCAGTGGTTTTGCGAC TCGTCTCCGGGYCTGTCTTT	DNA polymerase	560	Yes
NF-H1-F NF-H1-R	CCAAAGACCAAAGACCAG GTTGGTCTTTGGTCTCGCTC	Ranavirus Neurofilament-H1-like	639	Yes
FV3_79062F FV3_80059R	TTCTGTGTGCCCTGTACAACTGGA TCTTTGCGCGAGTGAGAAATGTGC	FV3 ORF 70R, 71R & 72L	998	Yes
FV3_-327009F FV3_327540R	AGGGCTACAAGGTCTGGCTTTCTT TTTACCATGGTGTCGAGCACCTCA	D5 family NTPase/ATPase	532	Yes

Methods/ Results

- Supernatant was prepared for EM, and virions were morphologically consistent with that of Ranaviruses
- DNA was extracted from infected BF-2 monolayers and amplified with a number of primer sets that amplify Ranaviruses. (Table 1).
- Specific genes targeted were the major capsid protein (MCP), DNA polymerase, neurofilament triplet H1-like protein (NF-H1) and D5 family NTPase/ ATPase. Another region of the genome that include putatuve genes of unknown function was amplified as well.
- The isolate was 100% identical to FV3 and a number of other related Ranaviruses across 475 bp of the MCP. No differences were noted in the sequences region of the DNA polymerase gene.
- Differences were, however, noted in the NF-H1 gene of the isolate and FV3. The sequence was 97% identical over 839 bp.
- The sequencing of 5 loci and over 3,200 bps revealed 99.1% identity between the turtle isolate and FV3.

Future Directions

- Pathogenicity studies are required to fulfill Koch's postulates and evaluate the comparative pathogenicity of this isolate to FV3 in an anuran host.
- Further sequencing is in queue to further distinguish this isolate from other Ranaviruses