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Identification of a Bohle iridovirus-like agent in captive North American boreal toads

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ABSTRACT

A “survival assurance” population of endangered boreal toads (*Anaxyrus boreas boreas*) experienced 93% mortality between April - July 2010. Toads were housed with other amphibians including some originating from Southeast Asia. Histology demonstrated lesions consistent with ranaviral disease including multicentric necrosis, vasculitis, and basophilic intracytoplasmic inclusion bodies. Initial confirmation of ranavirus (RV) infection was made by real-time PCR of a portion of the major capsid protein (MCP) gene and by demonstration of RV virions by transmission electron microscopy (TEM). Conventional PCR and sequencing of the MCP, DNA polymerase and NF-H1 genes demonstrated highest identity (86.3, 99.2 and 99%, respectively) with Bohle iridovirus (BIV). A virus, designated zoo ranavirus (ZRV), was isolated from infected toads and viral protein profiles, RFLP analysis and next generation DNA sequencing performed. Comparisons of ZRV genes (MCP and 18K) with other iridoviruses confirmed that ZRV was most similar to BIV. Although this is the first report of a BIV-like agent in North America, it is unclear if this virus is a novel North American BIV variant or acquired by exposure to co-housed amphibians. Moreover, several surviving toads remained PCR-positive 10 weeks after conclusion of the outbreak suggesting that ZRV is capable of establishing chronic infections and raising questions about the management of amphibians destined for reintroduction programs.

BACKGROUND

- The host range of most ranaviruses is uncertain.
- To date, BIV has only been detected in Australia.
- BIV naturally infects anurans, but is able to infect fish (barramundi) following experimental challenge.
- Examination of an outbreak of severe disease in a Midwestern zoo suggested that the causative agent was a ranavirus closely-related to BIV.
- Subsequent molecular analysis confirmed that a BIV-like agent, designated zoo ranavirus, triggered this die-off of boreal toads.

METHODS

- Histologic examination of tissues from affected toads
- Transmission electron microscopy
- PCR and sequence analysis of viral genes: MCP and 18K.
- Isolation of virus by standard ranavirus protocols
- SDS-PAGE analysis of viral protein profiles
- RFLP analysis of genomic DNA
- Whole genome sequencing and generation of phylogenetic trees

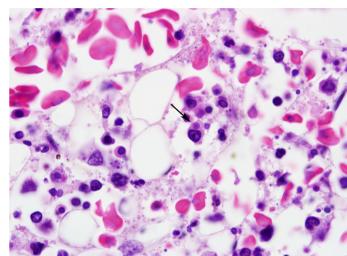
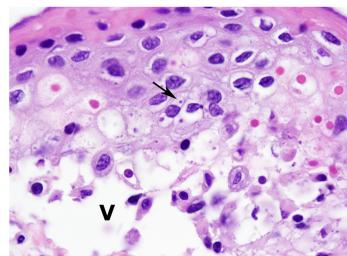


Fig. 1: Histological examination of skin (top) and bone marrow (bottom) show necrosis and intracytoplasmic inclusion bodies (arrows) suggestive of a ranavirus infection.

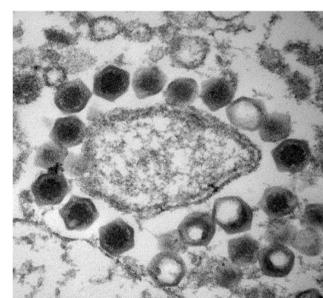
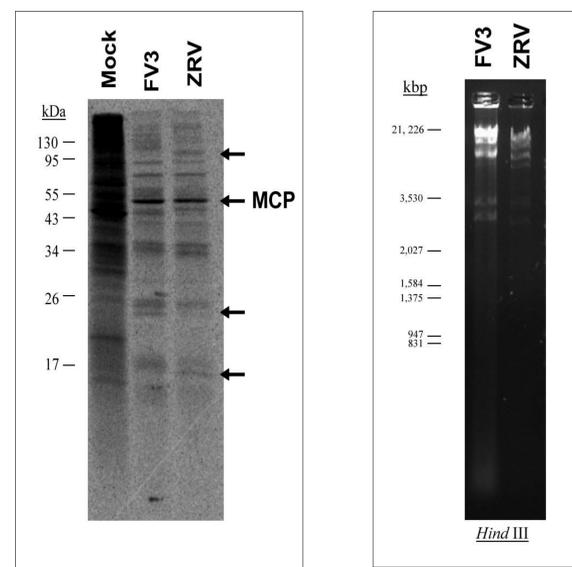


Fig. 2: TEM of a kidney from an infected toad demonstrates characteristic icosahedral virions ~150 nm in diameter.



Figs. 3 and 4: SDS-PAGE and RFLP analyses indicate that ZRV is likely a ranavirus similar to, but distinct from, FV3

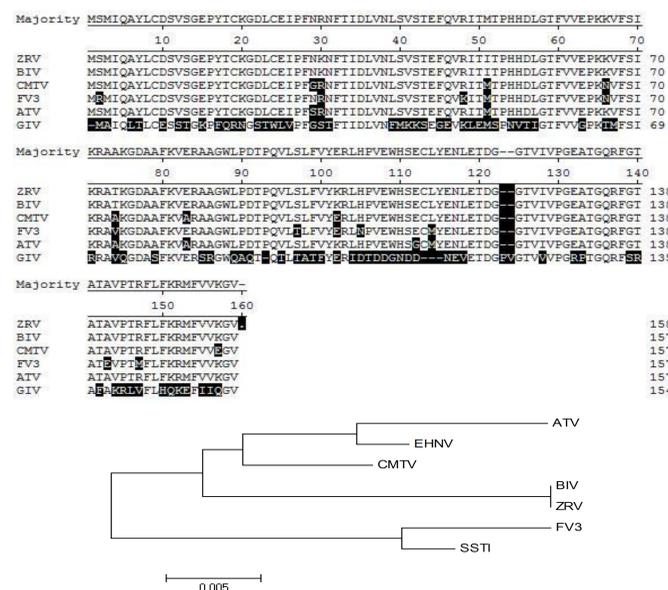


Fig. 5: Multiple alignment and phylogenetic analysis of RV 18K proteins. The ZRV 18K protein is identical to that from BIV. The alignment was generated using CLUSTAL W; the Neighbor-Joining tree using MEGA4.

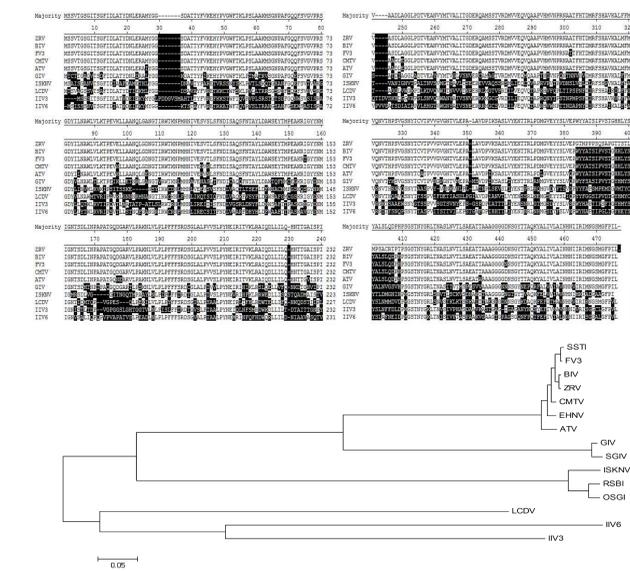


Fig 6: Multiple alignment (upper panel) and phylogenetic analysis (lower panel) of the ZRV MCP vs other iridoviruses confirm that ZRV is most closely related to BIV.

CONCLUSIONS

1. ZRV is a BIV-like virus based on microscopic, molecular, and genetic analysis.
2. It is not known if ZRV represents a novel North American BIV isolate or a virus acquired from one of the co-housed ectothermic species.
3. Sequencing of the complete genome of ZRV is ongoing and should provide additional information related to the taxonomic position of ZRV.
4. Persistence of viral DNA in surviving toads suggests that “healthy” animals may be infectious and serve as reservoirs of infection.