Assessing Blackworms as a Model for Studying Avian Vacuolar Myelinopathy

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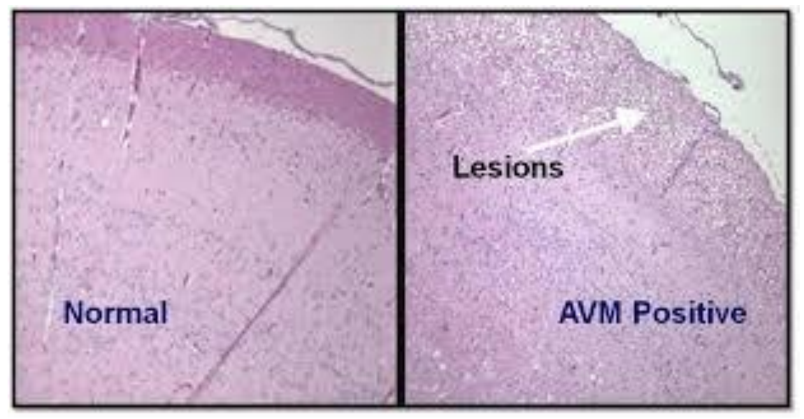
Introduction

- Avian Vacuolar Myelinopathy (AVM) is a fatal neurological disease that affects numerous species of birds in the Southeastern United States.
- The disease has been linked to a species of cyanobacterium (*Aetokthonos hydrillicola)* that grows on hydrilla, an aquatic plant. It is believed that this cyanobacterium produces a toxin which causes the disease when eaten.
- Although this disease has severe effects on birds, studies have shown that some species of fish, turtles and invertebrates are also susceptible to the toxin.

Bald eagles are one of the most common species affected by AVM
Photo: https://www.warnell.uga.edu/research/dr-susan-wilde-avm-research/history



AVM produces lesions in the brain. These photos display normal and AVM-positive mallard brains.
Photo: https://www.warnell.uga.edu/research/dr-susan-wilde-avm-research/history

- *Lumbriculus variegatus* (California blackworm) is a species of worm that inhabits shallow water environments such as marshes, swamps and ponds.
- They are often used as models in toxicity testing due to their low level of maintenance and cost efficiency.
- Blackworms reproduce asexually via fragmentation, and regeneration can be used as an endpoint in toxicity testing.

Objectives

- This series of experiments was done to test whether blackworms are a good model for testing the toxin that is believed to be responsible for AVM. Multiple endpoints were examined, including mortality, regeneration, and reproduction.

Collecting water and sediment samples at J. Strom Thurmond Lake

Methods

Exposure to Toxic Extracts

- Whole worms of approximately equal size were exposed to methanol extracts of hydrilla from AVM$^+$ and AVM$^-$ (control) locations
- Extracts were filtered prior to dosing using Amicon® Ultra Centrifugal Filters, 3kDa
- Worms (2 per treatment) were placed in 24 well plates in 2 mL spring water, plus extract (20 µl, 10 µl, 5 µl per well for each extract)
- 5 day exposure; mortality was assessed daily

Exposure to Environmental Samples

- Water and sediment samples were collected from Cherokee Recreation Area at J. Strom Thurmond Lake, GA (an AVM$^+$ location) on 11/11/2016, 12/2/2016, and 12/17/2016
- Control sediment was collected 01/14/2017 from Lake Hartwell, SC (an AVM$^-$ location). Spring water was used as a water control.

- Water Exposure Regeneration Tests
  - Head and tail regeneration was measured in each lake water sample and control
  - Head and tail segments of each worm (10 per treatment) were removed with a scalpel; tail and middle segments were counted
  - Middle segments were placed in a 24 well plate with 2 mL of treatment water
  - Regenerated segments were counted after 2 weeks and average number in each group was compared to controls via t-test. Worms are expected to grow back 8 head segments, regardless of the number removed. Tail segment regeneration is expected to match the number removed.
- Sediment Exposure Reproduction Test
  - Dried sediment (30 ± 1 g) from each collection date was mixed with dried nettle powder (food source) and placed into a glass jar. 100 mL of spring water was added.
  - 10 worms of approximately equal length were added to each jar
  - Water was constantly aerated over the course of the experiment and was partially exchanged with fresh spring water after 2 weeks
  - After 28 days, all worms were collected and counted

Results

- Exposure to Toxic Extracts
  - No mortality was seen at any concentration of AVM$^+$ or AVM$^-$ extract. Therefore, sublethal endpoints were assessed in the other experiments.
- Water Exposure Regeneration Tests
  - Average head regeneration was approximately the same in all groups. No significant differences were found between control and treatment worms (see Figure 1).
  - Regeneration of tail segments varied both within and between the groups (see Figure 2). Significant differences were found between the control and 11/11/16 treatment ($p = 0.03$), as well as the 12/17/16 treatment ($p < 0.001$). However, this experiment needs to be repeated due to a high death rate in the control group.

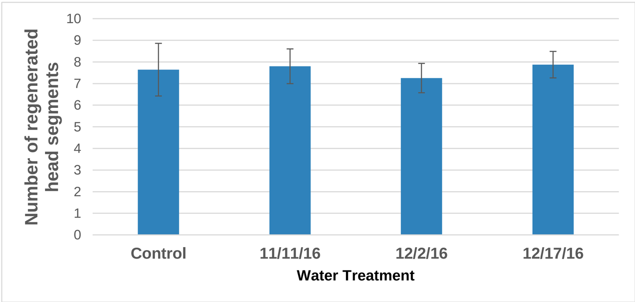


Figure 1. Average number of regenerated head segments in each treatment. Error bars show standard deviation.

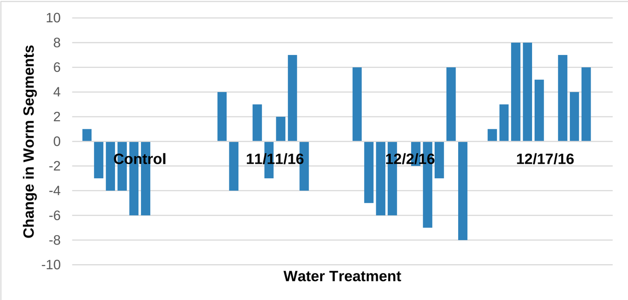


Figure 2. Difference in original versus regenerated worm tail segments in each treatment.

Results (Continued)

- Water Exposure Regeneration (Continued)
  - Two worms, one control and one Cherokee 12/17/16, grew abnormal head or tail segments (see Figure 3). Since only two such worms were observed, and one of them was in the control, this abnormality does not appear to be associated with the AVM toxin.
- Sediment Exposure Reproduction Test
  - The control sediment had the highest reproduction rate. Lake Thurmond water samples from earlier dates were found to have more worms than later dates (see Figure 4).

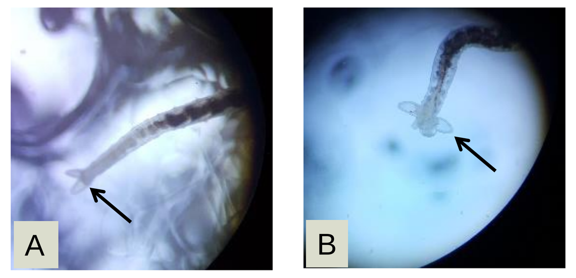


Figure 3. Abnormal segment regeneration in Cherokee 12/17/16 (A) and control (B) worms. Worm A has branched terminal head segments. Worm B has abnormal tail segmentation.

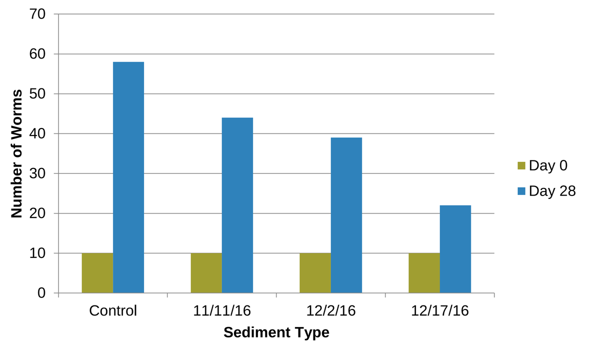


Figure 4. Number of worms in each sediment exposure at the beginning (day 0) and end of the experiment (day 28)

Discussion

- Blackworms do not appear to be acutely sensitive to the AVM toxin, as no mortality was seen in the experiments with toxic extracts.
- The head regeneration test does not seem to be a sensitive endpoint. The data from the test show that the average of the head segment regeneration between all four water treatments ranged around the same number.
- There was a significant difference in the tail regeneration but due to limited number of replicates and a high mortality rate additional testing must be done.
- In the sediment tests, fewer worms were collected in the lake sediments verses control. However, this test needs to be repeated to gain more replicates and test additional control sediment.

Conclusions

- From the tests done so far it is still difficult to determine whether blackworms are a good model for testing AVM. There were no deaths of intact worms in the water environments with the toxin nor was there evidence to support that the toxin affects their regeneration. The regeneration and reproductive tests need to be repeated, and additional tests with different endpoints should be conducted.

Acknowledgements

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