INTRODUCTION

Dracunculus spp. are spiroploric nematode parasites that live in the subcutaneous tissues or abdominal cavity of mammals. Female worms release L1 larvae into water when a host enters water and transmission occurs when another host ingests copepods infected with L3 larvae. The best known species is D. medinensis, the Guinea Worm. In 1985, over 3.5 million people were infected, but thanks to control efforts by the Carter Center and public health agencies, there were only 22 cases in 2015. Control was primarily through the use of water filters.

GOALS AND HYPOTHESES

Determine prevalence of Dracunculus in potential vertebrate hosts in Georgia

- H: Raccoons will be the primary host for Dracunculus spp.
- H: We will confirm that D. insignis is a host generalist.
- H: We will confirm that amphibians are suitable paratenic hosts.
- H: Fish have not been proven to be paratenic hosts but will be transport hosts.

Genetically characterize these worm to identify species

- H: We will confirm that D. insignis is a host generalist.

Experimental evaluate the role of paratenic hosts in the life cycle of D. insignis

- H: We will confirm that amphibians are suitable paratenic hosts.
- H: Fish have not been proven to be paratenic hosts but will be transport hosts.

MATERIALS AND METHODS

- Necropsy potential hosts and examine for Dracunculus spp.
- Extract DNA and perform PCR using primers LCO1490/HCO2198
- Sequence amplicons and conduct phylogenetic analyses
- Conduct experimental infection trials with tadpoles and fish
- Collect L1 larvae from live D. insignis and infect copepods
- Feed copepods L1 and after 14-18 days, feed to different species of tadpoles and fish
- Assess infection by necropsy and by feeding to ferrets, 6-8 months

RESULTS

Host-Parasite Associations

A total of 109 animals were necropsied and examined for Dracunculus. Only 2 species were positive: 25 of 51 (51%) raccoons and 5 of 39 (13%) opossums. Most had low worm burdens (<4), but one raccoon had 9 adult female worms. No male worms were found. Remaining examined hosts were negative.

Molecular Characterization

Amplifiers of the appropriate size were observed in numerous worm samples. Positive controls worked and negative controls had no amplification. However, sequencing results were problematic, due to difficulty sequence PCR reaction conditions and attempts to isolate DNA from a small portion of worm only. Repeated attempts to directly sequence purified products resulted in mixed products, vertebrate sequences, or a few Dracunculus sequences. The four parasite sequences were D. insignis.

Experimental Trials

L1 larvae were collected from a single female Dracunculus from a raccoon. Copepods were successfully infected as described.

Infections of 2 species of tadpoles (gray tree frog and northern cricket frog) confirmed by PCR. These frogs/parasites were fed to ferrets to confirm infectiosity. The host-fish indicate they are not likely paratenic hosts, we believe they could serve as transport hosts as we allowed fish to feed on infected copepods and then we immediately fed to ferrets. Due to the long development period of the parasite, results of these experiments will take 6-8 months.

FUN FACT

The Rod of Asclepius, a common symbol in healthcare, may reference the most commonly practiced method of removing the Guinea Worm from a patient. Female worms are slowly wound around a stick or gauze until the complete worm has been extracted.

DISCUSSION

Host-Parasite Associations

In a previous study in Canada, D. lutrae was only found in one host, the river otter, while D. insignis was a generalist, infecting raccoons, fishers, and muskrats. There are other studies that have found Dracunculus spp. (assumed to be D. insignis) in various hosts, but few look at sympatric hosts at the same site to investigate the role of various species in maintaining the parasite. In our study, as expected, raccoons were the most common host. Unexpectedly, we found that opossums were a relatively common host which represents only the 2nd report of Dracunculus in opossums. Although other hosts were negative, our sample sizes were low so continued surveillance is needed.

Molecular Characterization

Identification of Dracunculus spp. is easily done through the morphological characteristics of the adult male worm. Unfortunately, the male worm is rarely found. Identification of the female worms can be done through sequencing of DNA. Unfortunately, the primers previously used to identify species were not specific in our study. Many of the amplifiers submitted for sequencing showed amplification for the host DNA rather than the parasite DNA. Methods for better specificity and amplification and other gene targets of are currently being researched and results are pending.

Experimental Trials of Possible Paratenic Hosts

Despite excellent progress, D. medinensis is still a public health concern in 4 countries. Recently, the number of infected dogs, particularly in Chad, has increased. A possible explanation is the utilization of a paratenic host. We are conducting experimental trials with D. insignis as a model system for D. medinensis. Previously, burffows (Lithobates catesbeianus) and African clawed frog (Xenopus laevis) were experimentally shown to be possible paratenic hosts for D. insignis. Although most of our data are pending, we did confirm that two species of Hylidae frogs are susceptible. These frogs/parasites were fed to ferrets to confirm infectiosity. Finally, although previous work on fish indicate they are not likely paratenic hosts, we believe they could serve as transport hosts so we allowed fish to feed on infected copepods and then we immediately fed to ferrets. Due to the long development period of the parasite, results of these experiments will take 6-8 months.

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References