Brown Anoles (*Anolis sagrei*) as an Intermediate Host for the Invasive Pentastome Parasite (*Raillietiella orientalis*)

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Introduction

Raillietiella orientalis, an invasive pentastome parasite, was identified in several species of snakes native to Florida, indicating parasite spill-over from *Python bivittatus*[1].

The life cycles of pentastome parasites involve intermediate hosts and definitive hosts[2]. In an intermediate host, the pentastome larvae develop within the viscera to the infective stage[3]. As adults, pentastomes inhabit the respiratory systems of their definitive vertebrate hosts; typically, carnivorous reptiles[4]. We recently found high prevalence of *R. orientalis* in pygmy rattlesnakes at Lake Woodruff National Wildlife Refuge, 160 km North of the geographic range of *P. bivittatus*[5].

The spread of *R. orientalis* indicates that suitable intermediate hosts occur in Central Florida, but these hosts are currently unknown. Intermediate host species that are easily transported and abundant would facilitate the rapid growth of the pentastome's geographic range. To assess if brown anoles, *Anolis sagrei*, could be infected with *R. orientalis*, we exposed 21 individuals to viable eggs in a controlled experiment.

Hypotheses

1) Brown anoles, *A. sagrei*, can be infected with *R. orientalis* if exposed to viable eggs.
2) Infected *A. sagrei* will not exhibit adverse health effects (lower survival and growth).

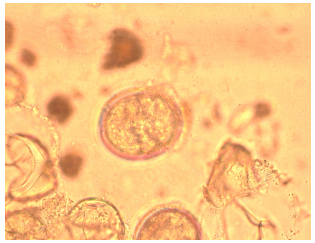

Figure 1. S. *miliarius* fecal sample with *R. orientalis* (400x).

Methods

Study Species: We collected 20 *Anolis sagrei* in DeLand, Florida. The lizards were kept in ventilated containers with a climbing branch, shelter, water, and a heat source.

Infection of Anoles: The control group was fed unaltered cricket fat bodies and the exposed group was fed fat bodies dusted with *R. orientalis* eggs. We chilled the anoles, which induced gaping when the anoles were handled and used a metal probe to place a cricket fat globule in the anoles' mouths.

Anole Husbandry: We misted the anoles daily, changed their waters daily, and cleaned their enclosures once a week. Every five days we fed the lizards three-six crickets.

Anole Dissection and Pentastome Records: The exposed and control lizards were euthanized and dissected between 5-168 days post treatment (under SU_IACUC-160).

Supplemental Anoles: To better estimate the latency between egg consumption and detectable larvae, we did additional trials with 11 lizards.

Data Analysis: We used a fisher exact test to determine if there was a difference between the frequency of infected anoles in the exposed and control group. We used a generalized linear model to determine the effect of the number of days between exposure and time of death, initial snout vent length, and pentastome treatment on anole growth. We determined the relationship between the number of days after exposure and the probability a lizard had detectable pentastome infection using a logistic regression model.

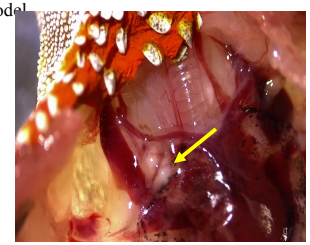

Figure 2. larvae of *R. orientalis* associated with the heart of an infected *A. sagrei*.

Figure 3. *Anolis sagrei* infected with *R. orientalis* larvae. The pentastomes were visible through the body wall.

Results

Exposure to pentastome eggs caused detectable infection with pentastome larvae in seven out of ten lizards. None of the control lizards appeared to be infected. Exposure to eggs significantly increased the likelihood of infection (fisher exact test, p= 0.0031).

The earliest larvae were seen in lizards at 75 days post exposure. Logistic regression indicated the probability of detectable pentastome infection significantly increased over the experimental period (χ^2=6.25, p=0.0124; Figure 4).

The cruciform larvae were found throughout the body cavities of the infected anoles and were associated with the dorsal and ventral body walls, liver, and heart. The larvae were loosely attached or deeply embedded in the tissues and approximately 0.6-0.7 mm in length (Fig. 2).

There was no significant difference in the growth rate between the exposed and control lizards (GLM, t=0.11, p=0.45; Figure 5). Lizards with shorter initial SVLs had significantly greater delta SVLs (t=3.05, p=0.0068; Figure 6).

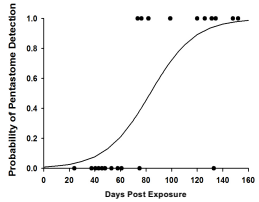

Figure 4. The probability of detecting larvae of *R. orientalis* in exposed *A. sagrei* over the 160 day experiment (n=21).

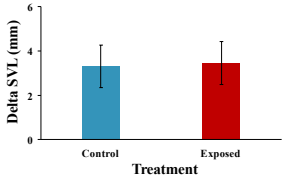

Figure 5. The mean change in SVL in lizards exposed to pentastome eggs and control lizards over time (n=10). ± 1 SEM.

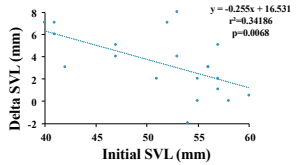

Figure 6. Initial SVL compared to delta SVL (n=20). Includes exposed and control lizards.

Conservation Implications

Our research indicates:

- Lizards in Florida are likely intermediate hosts for *R. orientalis*.
- The development to reach the infective stage appears to be approximately two months in captive *A. sagrei*.
- Infected lizards in this study did not exhibit adverse health effects.
- Anoles, which are often transported by humans, may be facilitating the rapid spread of *R. orientalis*.
- Snakes with lizard-rich diets including: *S. miliarius*, *Drymarchon couperi, Agkistridon spp., Coluber spp., Pantherophis spp., Thamnophis spp.,* and *Lampropeltis spp.* are likely at risk for pentastome infection[1].

The ability to be infected in captivity, however does not necessarily indicate *A. sagrei* is an important intermediate host in the wild. Further research on wild prevalence is needed.

Acknowledgements

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