PREVALENCE OF *PARELAPHOSTRONGYLUS ANDERSONI* IN WHITE-TAILED DEER, OTHER CERVIDS, AND BOVIDS AT A PRIVATE SANCTUARY IN NORTHERN FLORIDA

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INTRODUCTION:

Parelaphostrongylus andersoni, the muscleworm, is a nematode parasite that commonly affects white-tailed deer (*Odocoileus virginianus)* and has also been reported in barrenground caribou (*Rangifer tarandus (R. t.) groenlandicus* and *R. t. grantii*) and woodland caribou (*R. t. caribou*)[1]. *P. andersoni* infection has been noted to cause pulmonary lesions, eosinophilia, leukocytosis, caseous abscesses, and fibrous lesions[2,3]. It is prevalent in the Southeast United States, including Florida, and has been discovered in other states throughout the US[4,5]. The life cycle of *P. andersoni* involves the use of a gastropod intermediate host. After third stage larvae (L_3) are ingested by grazing deer, they migrate to the pulmonary system to mature. After maturation, first stage larvae (L_1) are excreted in the feces, where they infect nearby gastropods. The definitive host species and overall distribution of this parasite is still largely unknown at this time. The purpose of this study is to determine *P. andersoni* prevalence in Northwest Florida in white-tailed deer, a known definitive host, and if infection in other cervid and bovid species has occurred.

MATERIALS AND METHODS:

Feces (n=140) was collected from on ranch and 'off-site' animals. Feces was collected from numerous cervid and bovid species. Cervid species included: Fallow Deer (*Dama dama*, n=4), *O. virginianus* (n=47), Elk (*Cervus canadensis*, n=7), Sitka Deer (*Odocoileus hemionus sitkensis*, n=1), Axis Deer (*Axis axis*, n=6), and Père David's Deer (*Elaphurus davidianus*, n=14). Bovid species included: Nilgai (*Boselaphus tragocamelus*, n=5), Blackbuck (*Antilope cervicapra*, n=17), Scimitar Oryx (*Oryx dammah*, n=4), Gemsbok (*Oryx gazella*, n=9), and Jacob's Sheep (*Ovis aries,* n=1). After feces was collected it was stored at 4°C until simple sedimentation was performed. After sedimentation was completed, fecal pellet was assessed for presence of nematode larvae under a compound microscope. Remaining sediment pellet was stored at -20°C until DNA extraction and real-time PCR analysis.

Snails (n=34) were collected from around the ranch in areas that animal inhabitants had access too. Snails were identified after collection and processed. Snail species included: Squatty ambersnail (*Succinea unicolor*, n=31) and Rosy Wolf Snail (*Euglandina rosea (E.r.) bullata,* n=3). Live snails were digested in 10-50mL of 0.7% HCl and 3.0% pepsin. After digestion, larvae was recovered using the Baermann technique, and sample was assessed for presence of nematode larvae under a dissecting microscope. After assessment, sample was centrifuged to form a pellet and was stored at -20°C until DNA extraction and real-time PCR analysis. Dead snails were stored in ethanol until processing, where they were crushed and stored overnight at -80°C in a ATL Buffer solution until DNA extraction and PCR analysis was performed.

RESULTS:

RESULTS: Assessing presence of *Parelaphostrongylus andersoni* and *tenuis* in cervid, bovid, and snail species present at ElkHart Ranch. *Parelaphostrongylus andersoni* and *P. tenuis*[*] were verified using real-time PCR analysis. Fecal sedimentation was also performed to assess for nematode larvae

Cervid and Bovid Species:	N=	Nematode larvae presence in feces	*Parelaphostrongylus andersoni* presence
D. dama	4	1	0
O. virginianus	71	15	4
C. canadensis	7	0	0
O. hemionus sitkensis	1	0	0
A. axis	6	1	0
E. davidianus	15	0	0
B. tragocamelus	5	1	0
A. cervicapra	17	2	0
O. dammah	4	1	0
O. gazella	9	1	0
O. aries	1	0	0

* - real-time PCR results for *P. tenuis* still pending

Snail Species:	N=	Nematode larvae presence	*Parelaphostrongylus andersoni* presence
S. unicolor	31	0	0
E. r. bullata	3	3	0

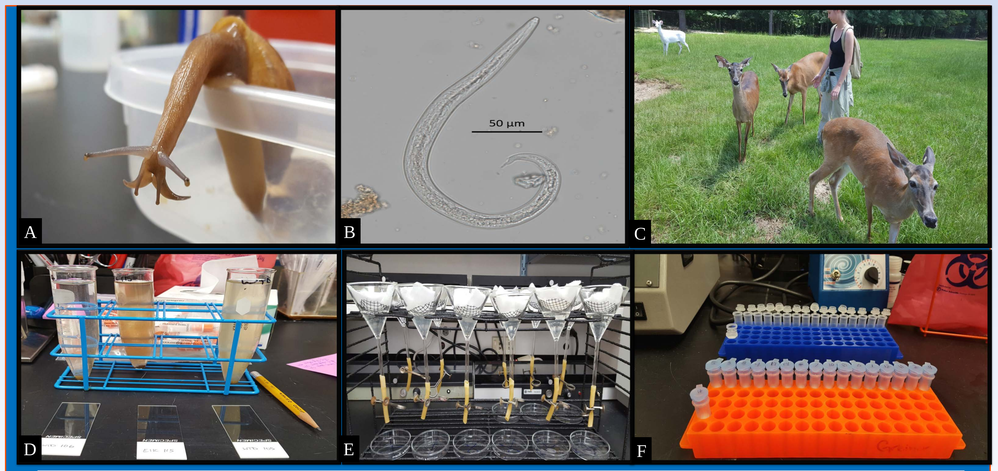

Figure 1: A) *Euglandina rosea bullata.* B) *Parelaphostrongylus andersoni* L_1. C) *Odocoileus virginianus* at the petting zoo at ElkHart Ranch. D) Fecal sedimentation set-up. E) Baermann Apparatus set-up. F) DNA extraction set-up

DISCUSSION:

Parelaphostrongylus andersoni, the muscleworm, has previously been identified in a limited number of hosts, including *O. virginianus*, *R. t. groenlandicus*, *R. t. grantii*, and *R. t. caribou*. Infection is noted to inflict musculoskeletal insult, such as weakness in the hindquarters and an altered gait. Larvae and egg presence in the lungs of infected white-tailed deer can produce pulmonary lesions which lead to granulomatous reactions resulting in localized interstitial pneumonia. Due to the damaging nature of this parasite, knowledge of *P. andersoni* presence in the environment is important for disease management[6]. Range of this parasite is most notable in the southeast United States, but has been found in varying states around the country. A small number of counties were assessed for its prevalence in those given states, and the study lacks a complete survey of the entire state. Further investigation into previously undocumented locations is important to map the spread of this parasite. This study expanded the search for *P. andersoni* in an undocumented area, Quincy, FL. Among incomplete topographical data of the spread of *P. andersoni*, the potential for other cervids and bovids to serve as definitive hosts remains unknown. Knowledge of all intermediate hosts involved is also unknown. Currently, we know infection to *P. andersoni* is due to deer accidentally consuming infected gastropods while grazing.

Parelaphostrongylus andersoni infection was not discovered at ElkHart Ranch, however it was identified in off-ranch *O. virginianus* (N=4) samples. No other species of cervids, bovids, or snails were identified with *P.* andersoni infection. Expanding the search in nearby areas is important to document the spread of this parasite in Florida.

ACKNOWLEDGEMENTS:

This research was funded by the Cervidae Health Research Initiative (CHeRI) and by the Merial Veterinary Scholars Program. Thank you to ElkHart Ranch for allowing us to utilize their facility for this project. Special thanks to these organizations for furthering the field of research.

REFERENCES:

1. **VEROCAI, G. G. et al.** A Nearctic parasite in a Palearctic host: Parelaphostrongylus andersoni (Nematoda; Protostrongylidae) infecting semi-domesticated reindeer in Alaska. Int J Parasitol Parasites Wildl, v. 2, p. 119-23, Dec 2013. ISSN 2213-2244.
2. **NETTLES, A. K. P. A. V. F.** Repeated Low-Level Infection of White-Tailed Deer with Parelaphostrongylus andersoni 1977.
3. **NETTLES, V. F.; PRESTWOOD, A. K.** Experimental Parelaphostrongylus andersoni infections in white-tailed deer. Vet Pathol, v. 13, n. 5, p. 381-93, 1976. ISSN 0300-9858 (Print) 0300-9858.
4. **INGRID M. ASMUNDSSON, J. A. M., AND ERIC P. HOBERG.** MUSCLEWORMS, PARELAPHOSTRONGYLUS ANDERSONI (NEMATODA: PROTOSTRONGYLIDAE), DISCOVERED IN COLUMBIA WHITE-TAILED DEER FROM OREGON AND WASHINGTON: IMPLICATIONS FOR BIOGEOGRAPHY AND HOST ASSOCIATIONS. Wildlife Disease Association: 12 p. 2008.
5. **ANNIE K. PRESTWOOD, V. F. N. A. F. E. K.** DISTRIBUTION OF MUSCLEWORM, Parelaphostrongylus andersoni, AMONG WHITE-TAILED DEER OF THE SOUTHEASTERN UNITED STATES. Wildlife Disease Association 1974.
6. **WOBESER, G.** Disease management strategies for wildlife 2002.