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Determining the Correlation Between Helminth Parasite Prevalence in Wisconsin Felis rufus Populations

Heather Gakev
Winona State University

Sara Eberhardt
Winona State University

Shalin Ranjitkar
Winona State University

Amber Schultze
Winona State University

Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Survey of Gastrointestinal Parasites in Bobcats in Wisconsin

Student Name Heather Gakev (Peters)

Faculty Sponsor Dr. Kimberly Bates

Department Biology

Abstract

Felis rufus (bobcat) populations are declining throughout some of their range in Wisconsin, while expanding in others (Personal Communication, WI DNR)1. Many theories exist as to why this is happening, including the possibility of disease. Our lab wanted to determine the helminth parasite population prevalence and intensity in Wisconsin bobcat and determine if there was a correlation with sex, age or geographic location of these animals. One hundred and fifteen bobcat intestines were collected March 5, 2012 at a Department of Natural Resources (DNR) Facility, Madison, WI. These animals were legally harvested by trapping/hunting and the carcasses were made available for scientific use by the WI DNR. Each intestine was analyzed by first washing the digesta through different pore sized screens or sieves, and then observing the retentate for parasites. Parasites were sorted and counted by species and gender and stored in 70% ethanol for further processing. Some parasites were stained or cleared and mounted for species determination. Currently, prevalence and intensity of infection are being determined and correlated with the geographic range, age, and gender of the animals.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature _____ Date _____

Faculty Sponsor Signature _____ Date _____

Determining the Correlation Between Helminth Parasite Prevalence in Wisconsin *Felis rufus* Populations

Sara Eberhardt, Shalin Ranjitkar, Heather Gakev, Amber Schultze, Douglas Gates, Alletia DeMartino, Kelsey Hoffmann, Josh Brandon, Jason Soderberg, Viktoriya Ishchuk, Selue Yang, and Abdi Gulaid

Dr. Kimberly Bates

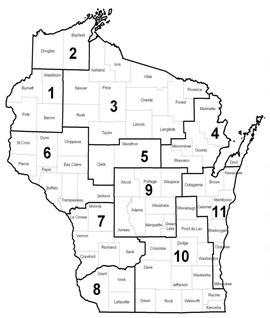
Biology Department. Winona State University, Winona, MN 55987

Abstract

Felis rufus (bobcat) populations are declining throughout some of their range in Wisconsin, while expanding in others (Personal Communication, WI DNR)[1]. Many theories exist as to why this is happening, including the possibility of disease. Our lab wanted to determine the helminth parasite population prevalence and intensity in Wisconsin bobcat and determine if there was a correlation with sex, age or geographic location of these animals. One hundred and fifteen bobcat intestines were collected March 5, 2012 at a Department of Natural Resources (DNR) Facility, Madison, WI. These animals were legally harvested by trapping/hunting and the carcasses were made available for scientific use by the WI DNR. Each intestine was analyzed by first washing the digesta through different pore sized screens or sieves, and then observing the retentate for parasites. Parasites were sorted and counted by species and gender and stored in 70% ethanol for further processing. Some parasites were stained or cleared and mounted for species determination. Currently, prevalence and intensity of infection are being determined and correlated with the geographic range, age, and gender of the animals.

Background

Bobcats (*Felis rufus*) are found mainly in Northern Wisconsin, with some found in Central and Southern Wisconsin. The main diet of bobcats are snowshoe hares, cottontail rabbits, and sick or old whitetail deer. Their secondary diet consists of porcupine, squirrel, woodchuck and mice. Bobcats may consume their secondary diet if their main food source is scarce or if there is preferred opportunity. These felines have an average life span of 15.5 years.



The habitat areas of the state of Wisconsin are determined by its DNR, based on the differing soil moisture and nutrient content in each of the areas. Bobcats were harvested from every county in habitats 2 and 3, with some collected from counties in habitats 1, 4, 6, 8 and 11. Each habitat supports different prey which may help determine the parasites bobcats are susceptible to.

The majority of bobcats that were collected lived in habitat area 3, which has dry-mesic and nutrient medium soil. The dominant tree types include sugar maple, hemlock, and beech. This creates the dense undergrowth that is favored by bobcats, and also their preferred prey of snowshoe hares and cottontail rabbits. This habitat also is favorable to the other rodents and deer that are the secondary source of food for bobcat populations.

Area collected in	Number of *F. rufis* collected
Habitat area 1	2
Habitat area 2	8
Habitat area 3	90
Habitat area 4	7
Habitat area 6	1
Habitat area 8	1
Habitat area 11	1

Due to their normal diet, parasitic relationships in bobcats can include parasites from the *Taenia*, *Toxocara*, *Toxascaris*, and *Diphyllobothrium* genus. *Taenia pisiformis* have a definitive host of cats and intermediate host of rodents; *Taenia pisiformis* uses dogs and cats as the definitive host and rabbits as their intermediate host. The definitive host for *Taenia rileyi* is specifically bobcat with rodents as intermediate hosts. *Diphyllobothrium* is also commonly found in bobcat intestines and generally use fish or frogs for the intermediate host and a carnivore as the definitive host. Both *Taenia* and *Diphyllobothrium* are classified as tapeworms. Bobcats may also host roundworms known as *Toxocara cati* and *Toxocaris leonine*, each with a definitive host of cats and intermediate host of small rodents.

Methods

Bobcat intestines were legally harvested on March 2nd, 2012 in Madison, WI from the DNR. A total of 115 intestines were collected by tying off the ends of the intestines and cutting them out. Each intestine was bagged individually, labeled with a number, and stored just below freezing temperatures to help preserve. In order to extract the parasites, the intestines were first thawed, cut lengthwise from the duodenum to the rectum, and the fecal matter was rinsed through four sieves, each with decreasing size pores. The smallest size pore diameter was 74 microns. The layers were collected into a beaker and the fecal-water mixture was examined through a light microscope. Unknown parasites were removed from the mixture and placed in marked vials with 70% ethanol. Each parasite was then distinguished individually and separated by family. Roundworms were identified by cervical alae characteristics and were classified as either *Toxocara cati* or *Toxascaris leonina*. Tapeworms were differentiated based upon morphology of proglottids and the scolex, if the scolex was present. They were classified as either *Taenia rileyi* or *Diphyllobothrium*.

All parasites were stored in 70% ethanol for preserving. If the parasites were stored in other types of solutions, they had to be rehydrated by transferring them into 70% ethanol for ten to twenty minutes.

Scolices were first stained with borax carmine (using only just enough to cover the parasites) for no longer than fifteen minutes. The parasites were transferred into a clean petri dish of astra blue stain (using just enough to cover the parasites) for about five seconds. They were then washed and rinsed with distilled water until the rinse was clear. Next, the samples were dehydrated in different concentrations of ethanol for about fifteen minutes each concentration, starting with 70% ethanol and increasing the concentration by 5%, until 100% concentration of ethanol was reached. Once dehydration of the scolices was complete, they were mounted with Canada balsam vector side up.

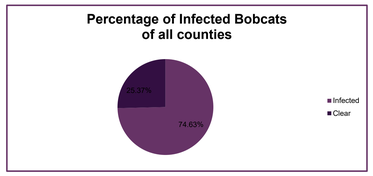
The latter, more mature, segments of the tapeworms were first stained with borax carmine, letting them sit for no longer than five minutes. The tapeworms were then rinsed until the proglottids appeared almost clear. After rinsing, the tapeworms were transferred into the coda blue stain, letting them sit for a maximum of five minutes. Once stained, the tapeworms were washed and rinsed until clear. The parasites were then dehydrated and mounted using the same procedure stated above.

Results and Discussion

After collecting and identifying the parasites, several variables were compared to find the correlation of helminth parasites and bobcat populations. Of these variables, county, age group, gender, helminth prevalence and type were analyzed.

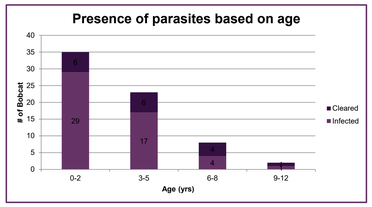
Figure 1 represents the percentage of infected bobcats in all counties. The taxonomic classification of the parasites in the bobcat intestines are not specified in this figure. It was determined 74.63% of the 68 bobcats were infected with parasites and the remaining 25.37% of the tested population were clear of parasites.

Figure 1



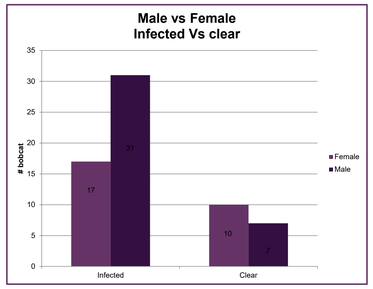
Each bobcat was aged using teeth ring data by the WI DNR. The age groups were zero to two; three to five; six to eight; and nine to twelve, as shown in Figure 2. In the age bracket of zero to two years old, 29 of the intestines were infected and six were clear. In the three to five age group, 17 infected and six clear intestines were found. The six to eight age group had the same parasitic load: four clear intestines and 4 infected intestines. There were two bobcat intestines dissected in the nine to twelve age group; one was infected and one was clear. Based on the collected data, the younger bobcat population is predicted to be more susceptible to parasitic infection compared to older adult bobcats.

Figure 2



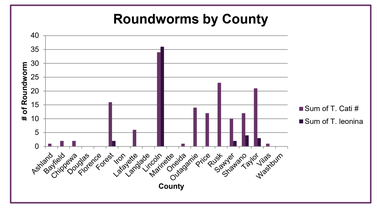
Of the 68 specimens analyzed, 48 intestines were infected and 17 were clear. Of the infected intestines, 31 were from male bobcats and 17 were harvested from females. In the clear intestines, seven were from female and 10 were from male bobcats. Female bobcats raise the young and stay in a relatively small area approximately 5 square miles, whereas males territories are variable in size and range from 20 to 30 square miles. According to these data, the males are more susceptible to parasitic infection than females possibly due to the range they travel in their habitat.

Figure 3



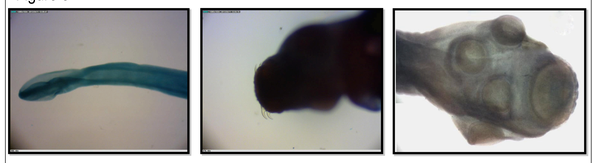
Roundworm prevalence and Wisconsin county data were measured, shown in Figure 4. There were two identified genus and species of roundworms: *Toxocara cati* (*T. Cati*) and *Toxascaris leonina* (*T. leonina*). Each were identified by the cervical alae characteristics.

Figure 4



Tapeworms were determined to be of the genus *Taenia* or *Diphyllobothrium*. Specific morphological features such as rostellum, rosettes and proglottid shape where used. Further identification of specific species is currently in progress. Figure 5 shows *Diphyllobothrium* scolex (left), prominent armed rostellum of *Taenia* (center), and unarmed *Taenia* rostellum with four suckers (right).

Figure 5



This is an ongoing project and future research opportunities are available. Further study on the collected tapeworm segments is piecing together the segments could help to identify the genus and species. Completing the 115 collected specimens and finishing these data would also aid in identification of the helminths.

Conclusion

1. Of the 68 dissected bobcat intestines, 74.63% were infected with parasites and the remaining 25.37% were not infected.
2. After determining the age ranges of collected bobcat intestines, it was found that the younger bobcat population ages zero to two and three to five were most susceptible to parasite infection.
3. From the 48 infected intestines, 31 of these were males and 17 were female. This indicates that male bobcats are more susceptible to parasitic infection.
4. *Toxocara cati* was found to be more prevalent throughout the counties with roundworm species than *Toxascaris leonina* except in Lincoln county where *Toxascaris leonina* was most prevalent.
5. Genus of tapeworms were based on their proglottids and visible rosette.

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Acknowledgements

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