

The Prevalence of Brain-Eating Roundworm *Baylisascaris procyonis* in Merrick County, Nebraska

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Summary

We conducted a study in Merrick County, Nebraska, to compare how the prevalence of *Baylisascaris procyonis* (raccoon roundworm) differs depending on the proximity of its host to human dwellings. We hypothesized that the prevalence and egg count of *B. procyonis* in *Procyon lotor* (raccoons) would be higher closer to human dwellings and that there would be more samples with parasites than without. To test these hypotheses, we performed necropsies and fecal flotation on 40 raccoon carcasses. 60% of the samples were positive for *B. procyonis*, and 40% were negative. We found that the disease was significantly more prevalent in areas closer to human dwellings. Although there was a small difference in the number of eggs between the groups, this difference was not statistically significant. More extensive testing should be conducted on the transmission of *B. procyonis*, as well as determining how prevalent it is in household pets and farm animals. Continued research of this nature is very important because *B. procyonis* can be lethal to humans. It is important to make the public aware of this disease, so precautions can be taken to avoid transmission.

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Introduction

Baylisascaris procyonis, more commonly known as raccoon roundworm, is a parasite that can be lethal to humans. Raccoons are the definitive host of this disease, but it is easily transmitted to dogs, cats, rabbits, woodchucks, and humans if the embryonated eggs are ingested (1, 2). The *B. procyonis* life cycle has several stages. In raccoons, eggs shed from adult worms exit through the feces. Larvae form within the eggs and the eggs containing larva can be transferred to humans, other raccoons, or smaller mammals. In

smaller mammals and humans, the larvae penetrate the gut wall, encyst in the visceral tissue, and continue to grow while in the host. In humans, the larvae migrate to a variety of tissues. Visceral larva migrans occurs when the roundworm larvae invade the visceral organs, and ocular larva migrans occurs when the roundworm larvae invade the eye (3). If the disease is transferred to raccoons, the adult worms begin to grow in the small intestine. The disease can be transferred to raccoons when they eat an alternative host or ingest embryonated eggs. As adult worms migrate through the host's body, they continuously grow and produce a large number of eggs, with an infected raccoon shedding an average of 17,527 eggs per gram of feces (4, 5).

B. procyonis is very prominent at raccoon latrines, places that multiple raccoons use to defecate over long periods of time (4). Raccoon latrines can be found in many areas that are associated with human dwellings, including sandboxes, rooftops, chimneys, decks, and outbuildings, which can increase the likelihood of human infection (2). Large numbers of eggs are typically more prevalent at raccoon latrines. Depending on the environment, *B. procyonis* eggs can stay viable for up to two years, which can also increase the likelihood that humans become infected (6).

The threat of *B. procyonis* to human health should be taken seriously, as several studies have shown its prevalence in raccoon populations. One study found *B. procyonis* eggs in close proximity to human dwellings (8). In the United States, *B. procyonis* is prevalent in the western and southern states (2). Studies done in North Carolina, Wyoming, and Southern Texas all reported the prevalence of *B. procyonis* in raccoons (2, 8, 9). However, much of the data collected on *B. procyonis* is over 30-50 years old (2), and after a comprehensive literature search, it is apparent that more research is needed to determine the prevalence of the parasite in the Midwest area of the United States. *B. procyonis* is an emerging public health concern in North America (2), and one of the many zoonotic diseases of which the public needs to be aware.

Our objective was to compare the difference in prevalence of *B. procyonis* in Merrick County, Nebraska between areas in close proximity to human dwellings and areas farther from human dwellings through



Figure 1. Micrograph of *B. procyonis* eggs

necropsy and fecal examination of collected samples. Research of this nature is of vital importance because man-made changes in the environment have caused higher raccoon densities in certain areas associated with humans. Raccoons travel through and inhabit public parks, neighborhoods, and the backyards of humans, and a greater understanding of the parasite's distribution may help limit disease transmission to humans.

We hypothesized that the prevalence and egg count of *B. procyonis* in *Procyon lotor* (raccoon) would be higher near human dwellings, because raccoon latrines are more common in these areas (4). Furthermore, we hypothesized that there would be more samples with parasites than without, because previous studies have shown a prevalence of *B. procyonis* near or above 50% (2, 9). We performed necropsies and fecal flotation on 40 raccoon carcasses from various locations, counting the number of *B. procyonis* from each sample. We found that although a majority of samples were positive for *B. procyonis*, there was a significant difference in prevalence according to location.

Results

Raccoons that had been killed by permit-bearing trappers, vehicles, or rural land owners for eradication purposes were collected in Merrick County, Nebraska, and were tested for *B. procyonis* to gain a greater understanding of the parasite's distribution in order to help limit transmission to humans. Of these carcasses, 19 were located in close proximity to human dwellings (CPHD), and 21 were from areas not in close proximity to human dwellings (NCPHD). Necropsies were performed on 40 carcasses, the contents of the small intestine were extruded and examined, and a fecal sample was obtained from each carcass. We used the fecal flotation method on each fecal sample to determine the presence of *B. procyonis*, and if the parasite was present, we counted the number of eggs on a sample slide (Figure 1). Of the

40 samples tested, 60% were positive and 40% were negative. There were 46% more positive samples from areas that were in close proximity to human dwellings compared to areas that were not (Figure 2). A Chi-square test showed a significant difference in prevalence according to location ($p=0.002$), with the disease having higher prevalence in areas closer to human dwellings. An average of 20 more individual eggs were found in the samples taken from areas that were not in close proximity to human dwellings compared to those that were in close proximity to human dwellings (Figure 3). A t-test showed there was no significant difference in the number of eggs between the two groups ($p=0.7$).

Discussion

The purpose of this study was to determine the prevalence of *B. procyonis* in Merrick County, Nebraska, and to determine if there was a difference in the presence of the disease depending on the location of the raccoons relative to human dwellings. In concordance with our hypothesis, a higher number of the samples were positive than negative. This may have been because Merrick County is located in a central region of United States, and numerous species such as woodchucks, skunks, opossums, and foxes that can be hosts to *B. procyonis* are prevalent in the region. Many of these species live near both urban and rural human dwellings, which leads to frequent interactions with humans.

Because there was a statistically significant difference in *B. procyonis* prevalence according to location, raccoons that roam in close proximity to human dwellings may be more likely to be hosts to *B. procyonis*. (2). There may have been more positive samples in areas close to human dwellings because as raccoons

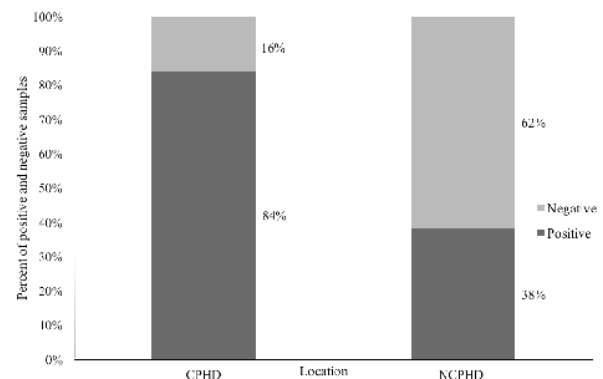


Figure 2: Percent of positive and negative samples from areas in close proximity to human dwellings (n=19) vs. areas not in close proximity to human dwelling (n=21). A Chi-square test showed a significant difference in prevalence according to location ($p=0.002$), with *B. procyonis* having higher prevalence in areas closer to human dwellings. NCPHD—areas not in close proximity to human dwellings; CPHD—areas in close proximity to human dwellings.

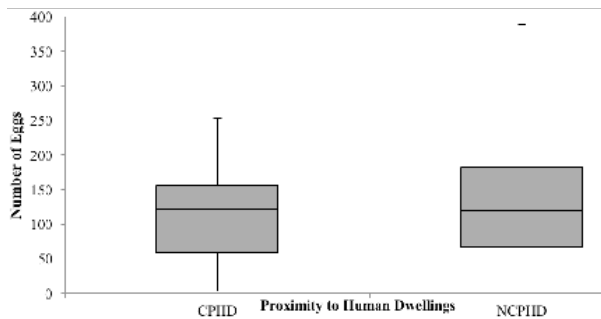


Figure 3: Number of eggs in samples from areas in close proximity to human dwellings (n=19) versus areas not in close proximity (n=21). A t-test showed no significant difference in the two groups (p=0.7). CPHD-close proximity to human dwellings; NCPHD-not in close proximity to human dwellings.

Sample #	Location	Positive	Negative	Egg Number
1	NCPHD	x	Negative	x
2	NCPHD	x	Negative	x
3	NCPHD	x	Negative	x
4	NCPHD	Positive	x	22
5	CPHD	Positive	x	186
6	NCPHD	x	Negative	x
7	NCPHD	x	Negative	x
8	NCPHD	x	Negative	x
9	CPHD	Positive	x	3
10	CPHD	Positive	x	50
11	CPHD	Positive	x	123
12	NCPHD	Positive	x	180
13	NCPHD	x	Negative	x
14	NCPHD	x	Negative	x
15	NCPHD	x	Negative	x
16	NCPHD	x	Negative	x
17	NCPHD	x	Negative	x
18	CPHD	Positive	x	34
19	NCPHD	x	Negative	x
20	CPHD	Positive	x	253
21	CPHD	x	Negative	x
22	NCPHD	Positive	x	75
23	CPHD	Positive	x	118
24	NCPHD	x	Negative	x
25	CPHD	Positive	x	127
26	NCPHD	Positive	x	72
27	CPHD	Positive	x	98
28	NCPHD	Positive	x	162
29	CPHD	Positive	x	58
30	CPHD	Positive	x	87
31	CPHD	x	Negative	x
32	CPHD	Positive	x	237
33	CPHD	x	Negative	x
34	CPHD	Positive	X	146
35	CPHD	Positive	x	58
36	NCPHD	Positive	x	187
37	NCPHD	Positive	x	47
38	CPHD	Positive	x	129
39	CPHD	Positive	x	389
40	NCPHD	Positive	x	239
TOTALS:	+CPHD=84% +NCPHD=38%	-CPHD=16% -NCPHD=62%	F=60% N=40%	CPHD=122 NCPHD=142

Table 1: *B. procyonis* prevalence and egg number from areas not in close proximity to human dwellings (NCPHD) and areas in close proximity to human dwellings (CPHD).

travel through urban areas looking for shelter and food, they may be more likely to come into contact with other raccoons, thereby increasing the chance of disease transmission. There was a small difference in the number of eggs between the groups, but the difference was not found to be statistically significant, so the hypothesis that there would be a difference in the number of eggs according to location was not supported.

Although differing study locations make it difficult to directly compare the results of the current study to previous studies, the current study does show a high prevalence of *B. procyonis* in raccoons, which aligns with the results of some earlier studies. In one study in Southern Texas, 70% of the samples examined contained *B. procyonis* (9), while in the current study, 60% of the samples were positive. A study of raccoons in Wyoming found that 45% of the samples tested between 2009 and 2011 were positive for *B. procyonis* (2), and other researchers found a 12% prevalence in a study in North Carolina in 2012 (4). It will be important to conduct more studies on the prevalence of *B. procyonis* in Central Nebraska to provide a direct comparison for the current study.

Because this study was the first to be conducted on *B. procyonis* in Nebraska, repetition and expansion of this research is needed. More extensive testing on the transmission of this parasite will aid in the development of a comprehensive plan for preventing human infection. Information on the prevalence of *B. procyonis* in household pets and farm animals could be beneficial to the public to help limit transmission. Also, determining the viability of the eggs in different soil temperatures is important, because as global temperatures continue to rise, prevalence of this parasite may increase. Therefore, a next step may be a long-term study investigating whether there is a correlation between climate and *B. procyonis* prevalence. Because of the deadly nature of *B. procyonis* and the close relationship between humans and raccoons, more research is needed to determine the prevalence of *B. procyonis*, particularly in the Midwest area of the United States.

Methods

Fecal sample collection

Raccoons that had been killed by permit-bearing trappers, vehicles, or rural land owners for eradication purposes were collected in Merrick County, Nebraska. Eye goggles, a breathing respirator, and gloves were worn before working with the samples. Necropsies were performed on 40 carcasses, the contents of the small intestine were extruded and examined, and a fecal sample was obtained from each carcass.

Fecal flotation method

The fecal flotation method was used on each fecal sample to determine the presence of *B. procyonis*. If the parasite was present, the number of eggs was counted on a sample slide. During the fecal flotation process, a portion of the sample was mixed in Sheather's solution and poured through a strainer into a centrifuge tube. The sample was centrifuged at 1,100 rpm for ten minutes. To determine if a sample was positive for *B. procyonis*

eggs, images from Dr. Michael Dryden of Kansas State University were used for comparison, and all 40 samples were sent to Kansas State University, College of Veterinary Medicine, Parasitology Department, for verification.

Data analysis and statistics

Data were organized by location, separating the data from rural areas and areas in close proximity to human dwellings. Close proximity was designated as within five miles of a human structure. The percent of fecal samples that tested positive for raccoon roundworm was calculated. A Chi-square test on prevalence was run to determine whether the null hypothesis could be rejected. A t-test was run to determine whether there was a difference in the number of eggs between the two groups.

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