Distribution and prevalence of *Baylisascaris procyonis* in raccoon populations in Allegan and Barry Counties, Michigan

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Abstract

Baylisascaris procyonis (raccoon roundworm) is a species of ascarid worm that lives within the intestinal tract of the common raccoon (*Procyon lotor*). Raccoons typically defecate in communal areas, called latrines. Millions of *B. procyonis* eggs can accumulate at latrine sites and can remain infective for years. Many animals, including humans, can serve as intermediate hosts for raccoon roundworm. Ingestion of the eggs can result in ocular, neural or visceral migrans which can lead to brain damage and, in some cases, death. Our goal was to determine the distribution and prevalence of raccoon roundworm in Allegan and Barry Counties in Michigan. We sampled scat from latrine sites and also necropsied raccoon road kill to look for the presence of *B. procyonis*. A total of 191 fecal samples and fifteen dead raccoons were collected from Allegan and Barry Counties. A 5.1% prevalence rate was found for Barry County and a 5.5% prevalence rate was found for Allegan County. These prevalence rates are fairly low; however, we did note that there was spatial variability between among sample locations. The necropsy results yielded a prevalence rate of 33%, indicating there may be a seasonal component for when the adult worms shed their eggs. Also shedding of eggs in many helminthes can be intermittent and inconsistent from day to day, so one scat pile from a single raccoon may have a lot of eggs and the next may not. There can also be pre-patent infestations – raccoons infected with *B. procyonis* that are not yet mature enough to produce eggs – or the number of worms in an individual may be low, reducing the likelihood of finding eggs in any one scat sample. Thus there could be false negatives from the fecal samples, a possible explanation for our differing prevalence rates.

Introduction

Baylisascaris procyonis (also known as raccoon roundworm) is a parasitic ascarid nematode that can be found in the intestinal tract of the common raccoon (*Procyon lotor*). *B. procyonis* is zoonotic, causing visceral, ocular and neural larval migrans in humans and wildlife if ingested (Gavin *et al.* 2005; Roussere *et al.* 2003). Cases of human infection are most common in children and individuals suffering from pica or geophagia (Sexsmith *et al.* 2009; Page *et al.* 2008).

Transmission of *B. procyonis* to its raccoon hosts can occur via two different routes and has been shown to be age-dependent (Roussere *et al.* 2003). Juvenile raccoons become infected directly by ingesting contaminated eggs from their den or nearby latrine sites while scavenging for seeds in infected feces, during investigative behavior, or during communal grooming activities. Adult raccoons can only become infected by consuming intermediate hosts infected with *B. procyonis* (Gompper and Wright 2005; Kazacos 2001; Roussere *et al.* 2003; Yeitz *et al.* 2009). Once infected, raccoons shed eggs at rapid rates, ≈ 115,000-179,000 eggs/worm/day, through their feces (Gavin *et al.* 2005). Raccoons typically defecate in communal latrine sites which accumulate feces from multiple individuals (Page *et al.* 2009; Kazacos 2001; Roussere *et al*. 2003). This social behavior, paired with a high infection rate (up to 86% in Illinois, 74% in Indiana, 25% in Ohio, and 58% in Michigan), creates both public health concerns and environmental issues due to the fact that latrines harbor millions of eggs that can remain infective for years (Kazacos and Boyce 1989; Kazacos 2001; Murray and Kazacos 2004). .

The rate of raccoon infection has been observed to be positively linked with densities of intermediate hosts (Gompper and Wright 2005). More than ninety mammal and bird species have been identified foraging in and around raccoon latrine sites in search of undigested seeds, resulting in accidental ingestion of infected eggs (Page *et al.* 1999; Roussere *et al.* 2003; Yeitz *et al*. 2009). Common intermediate hosts include small mammals, such as white-footed mice (*Peromyscus leucopus*), tree squirrels (*Sciurus carolinensis*), and eastern chipmunks (*Tamias striatus*), as well as many species of birds (Evans 2002; Page *et al.* 1999). When ingested by an intermediate host, larvated eggs hatch in response to the low pH levels of the stomach. The larvae burrow through the small intestine and begin to 'wander' through host tissue (i.e., larval migrans). Larval migrans may cause central nervous system diseases which can increase the likelihood of predation (Murray and Kazacos 2004). If adult raccoons ingest the infected intermediate hosts, they become infected and the raccoon roundworms stay in the intestines absorbing nutrients and shedding eggs, thus continuing the life cycle of the roundworm.

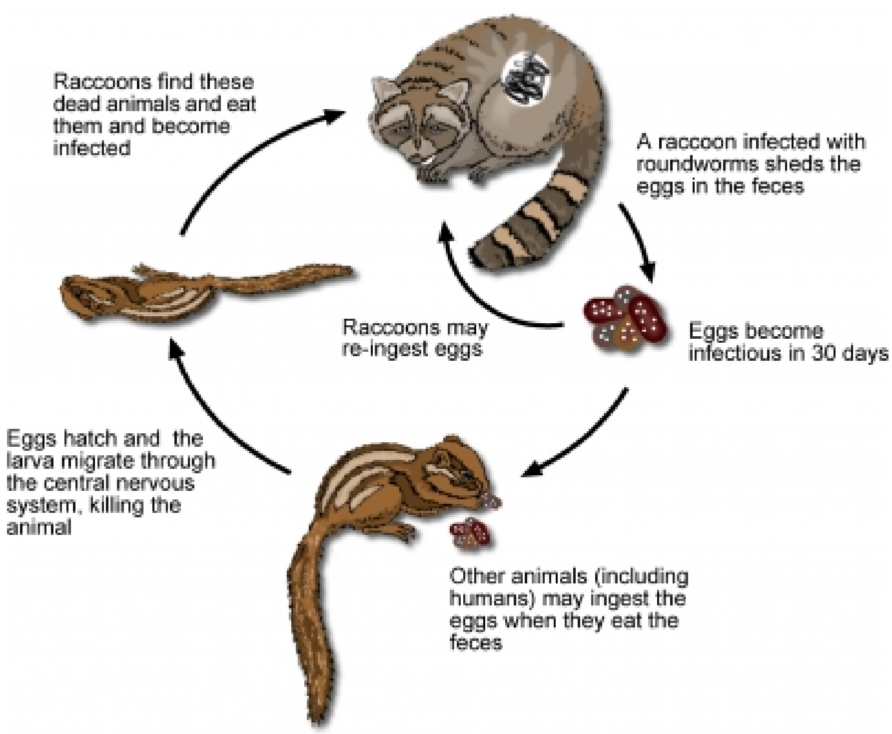


Figure 1: Life cycle of *Baylisascaris procyonis* (raccoon roundworm) through the intermediate host route. Source: Purdue Extension Entomology extension.entm.purdue.edu

Acquiring information on the distribution and prevalence of infected raccoons is the first step in creating a management plan (i.e., prevalence being defined as the number of infected individuals/total number sampled). Because raccoons have the ability to adapt to urban environments and habituate themselves readily to human activity, they can have latrine sites in close proximity to human developments, which poses a public health risk (Samson *et al.* 2012; Sexsmith *et al.* 2009). Latrine sites can accumulate millions of *B. procyonis* eggs, which are extremely durable and can resist changes in pH, desiccation, temperature extremes, and cannot be killed by routine disinfectants. The primary method to decontaminate an area is to raise the temperature above 62 °C by using a flame torch or using a steam producing device (Shafir *et al.* 2011). The desensitization of raccoons to human activity, potentially high infection rates of raccoons, high viability of raccoon roundworm eggs and potentially fatal health hazards make *B. procyonis* an important area of study.

During the summer of 2012, Michelle DeMuro and Michael Rossi performed an intensive survey of the property of Pierce Cedar Creek Institute (PCCI) in Barry County to identify raccoon latrines and sample them for the presence of raccoon roundworm (Rossi *et al.*, in prep). They documented over 100 latrine sites and tested over 300 samples for raccoon roundworm and found a 0% prevalence rate. During the fall of 2012, 30 raccoons obtained from Kent and Ottawa Counties were necropsied. Nineteen of those individuals harbored raccoon roundworm, a 62% prevalence rate (Professor Joe Jacquot and Professor Doug Graham, GVSU, personal communication). We think there are two possible reasons for this discrepancy. First, there may be a difference in sensitivity of the two methods in detecting raccoon roundworm. Detection from raccoon latrines is dependent upon infected individuals shedding eggs in detectable quantities, whereas necropsy results are definitive (Page *et al.* 2005). Raccoon roundworms may not be mature enough to shed eggs and there may also be a seasonal component for when the adult worms shed their eggs. Page *et al.* (2005) suggests that although latrine sampling is not as good at detecting raccoon roundworm, it is sensitive enough to give reliable results. Second, Pierce Cedar Creek Institute may simply not have had any raccoons infected with raccoon roundworm present in May – August 2012.

For our project, we sampled a broader geographic area, focusing on Barry and Allegan Counties. PCCI was intensively sampled again to determine whether the prevalence of raccoon roundworm changed over the year from 2012 to 2013. We sampled raccoon latrines and performed necropsies over the two county area, hypothesizing that we would find a prevalence similar to the average of 60-80% for the Midwest (Page *et al.* 1999; Page *et al.* 2008) We also selected a subset of raccoon roundworm positive and negative latrine sites and measured intermediate host densities at those sites to test the hypothesis that intermediate host density is linked to raccoon roundworm prevalence. Track plates were used to index local small mammal population densities (Conners *et al.* 2005). We hypothesized that raccoon roundworm positive latrine sites would have higher densities of intermediate hosts than raccoon roundworm negative latrine sites, based on the results of previous studies (Page *et al.* 2001a; Page *et al.* 2001b). Data was utilized to estimate the level of public health risk at PCCI, the rest of Barry County, and Allegan County.

Methods and Procedures

We systematically searched for latrine sites and collected fecal samples from Allegan and Barry Counties in Michigan during May-July 2013. We had five sample locations in each county, with PCCI being one sample location in Barry County. Latrines were defined as single or multiple scats located less than five meters from other scats and were found on fallen logs, on rock piles, on the ground and at tree bases (Page *et al.* 1998). Once located, latrines were flagged and GPS coordinates were recorded. Only active latrine sites were sampled, and the fresh feces were tested for the prevalence of *B. procyonis* eggs. The eggs take about 2 - 4 weeks to become infective once they are shed, making fresh feces significantly safer to handle (Murray and Kazacos 2004). Collected scat samples were stored in Ziploc bags and frozen at -20 ° C until tested. Freezing does not affect the viability of *B. procyonis* eggs, and so does not bias our ability to detect their presence (Shafir *et al.* 2011).

Fecal flotation was performed to detect *B. procyonis* eggs, using a modified detergent wash flotation procedure with Sheather's sugar solution (specific gravity 1.25-1.27) and a centrifuge. Approximately 2 grams of feces from each sample was mixed with 10 mL of Sheather's sugar solution. This mixture was strained through cheesecloth to remove any large debris and was poured into test tubes. The test tubes were then centrifuged for 5 minutes, after which the test tubes were filled with sugar solution until there was a reverse meniscus. Coverslips were placed over the test tubes for 10 minutes so that any parasites found in the feces (including *B. procyonis* eggs) could adhere to the coverslip. After 10 minutes, the coverslips were transferred to microscope slides (Dryden *et al.* 2005). Entire slides were examined for *B. procyonis* eggs using a light microscope at 100X. All utensils and counters were wiped down with an all-purpose cleaning solution, which removes the eggs' adhesive coat and aids in surface removal.

Necropsy was another method used to determine the prevalence of *B. procyonis* in the raccoon populations of Allegan and Barry County. Fresh road-killed raccoons were obtained from Allegan and Barry Counties and GPS coordinates were recorded. For examination, a midline incision was made from the xiphiod process to the pubis bone. At this point, the gastrointestinal tract was removed and fecal samples from the colon were acquired. The gastrointestinal tract was also examined for the presence of adult worms (Yeitz *et al.* 2009). Examination of carcasses occurred in the lab at PCCI; however, the carcasses were bagged, frozen, and disposed of at Grand Valley State University. Prevalence in raccoon populations was estimated in two ways, as the proportion of (1) fecal samples and (2) individuals recovered from roads that are found to be infected from the sampled populations.

After the samples were collected and analyzed, we found that Charlton Park in Barry County had a number of raccoon roundworm positive samples. Therefore, we selected 5 raccoon roundworm positive latrine sites and five negative latrine sites that were similar in terrain and undergrowth density at Charlton Park for our track plate study. Five negative sites were also selected at PCCI as another control. We used the track plates to determine the level of intermediate host activity in and around the latrine sites. Specifically, we focused on the activity of the eastern chipmunk, white-footed mouse, and tree squirrel as these are the three most common intermediate hosts that have been found to visit latrine sites; however, any activity of other potential intermediate hosts was also recorded (Page *et al.* 1999).

Track plates were made by applying an 80% ethanol, 15% powdered graphite and 2.5% mineral oil mixture to acetate sheets (following the methods of Connors *et al.* 2005). Once the sheets were dry, they were adhered to aluminum flashing, which served as a backing for the sheets to increase their durability. Five track plates were placed at each selected latrine site with each track plate being no more than two meters away from the latrine site. Once placed, track plates were checked every 24 hours and any containing tracks were removed and replaced with a fresh track plate. The track plates were placed at the selected latrine sites in early August 2013 for a total of five days. The number of visits a latrine received from possible intermediate hosts was quantified as the total number of tracks and as the number of track plates with any tracks. A One-way ANOVA was used to analyze and interpret the results from the track plates.

Results

Overall, we collected and processed a total of 137 scat samples from Barry County and 54 scat samples from Allegan County. Of the samples collected from Barry County, seven tested positive for raccoon roundworm, yielding a 5.1% prevalence rate. Within Barry County, we found differing prevalence rates at each of the five sites (Table 1). From the samples found in Allegan County, three of them were positive for raccoon roundworm. This gives Allegan County a 5.5% prevalence rate. Allegan County also had varying prevalence rates among the five sites (Table 2). Other things found in the fecal floats included monocystis (an endoparasite commonly found in earthworms), soil nematodes, pollen grains, and plant spores (Figures 4 and 5).

Of the fifteen raccoons we necropsied, only one of them was recovered from Allegan County while the other fourteen were recovered from Barry County. Eight of the necropsied raccoons were males and seven were females. We had a total raccoon roundworm prevalence rate of 33% from our necropsies, with the majority of the infected individuals being juveniles (Table 3).

For track plate analysis, we used a One-way ANOVA to determine if there were differences in the amount of visitations to the positive and negative latrine sites. Analyzing the number of raccoon visitations at each latrine site gave a p-value of 0.705 with df = 2 and F = 0.36 (Figure 6). Raccoon visitations between positive and negative latrines sites were not significantly different; however, there were more raccoon visitations at Charlton Park than there were at Pierce Cedar Creek Institute. The intermediate hosts, which included tree squirrels, white footed mice, and eastern chipmunks, had a p-value of 0.008 with df = 2 and F = 7.38 (Figure 7). There were more intermediate host visitations at Charlton Park than there were at Pierce Cedar Creek Institute.

Table 1: Prevalence of *Baylisascaris procyonis* (raccoon roundworm; RRW) at sample locations in Barry County, Michigan.

Sample Locations	Total Scat Samples Collected	RRW positive	Prevalence rate (%)
PCCI	65	1	1.54
Charlton Park	25	6	24.0
Barry State Game Area	8	0	0.0
Yankee Springs Recreational Area	38	0	0.0
Middleville State Game Area	1	0	0.0

Table 2: Prevalence of *Baylisascaris procyonis* (raccoon roundworm; RRW) at sample locations in Allegan County, Michigan.

Sample Locations	Total Scat Samples Collected	RRW positive	Prevalence rate (%)
Little John Lake Park	2	0	0.0
Dumont Lake	13	1	7.7
Saugatuck Dunes State Park	8	0	0.0
Allegan State Game Area	22	1	4.6
Silver Creek County Park	9	1	11.1

Table 3: Prevalence of *Baylisascaris procyonis* (raccoon roundworm) in necropsied raccoons from Barry and Allegan Counties in Michigan.

		Percent Infected (%)
Male (8)	Adult (3)	33.0
	Juvenile (5)	60.0
Female (7)	Adult (4)	0.00
	Juvenile (3)	33.0

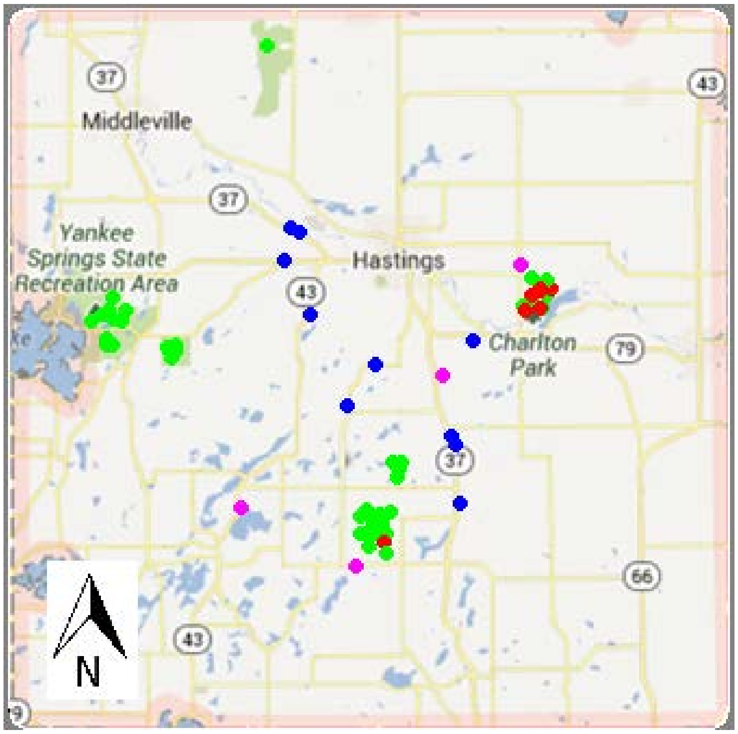
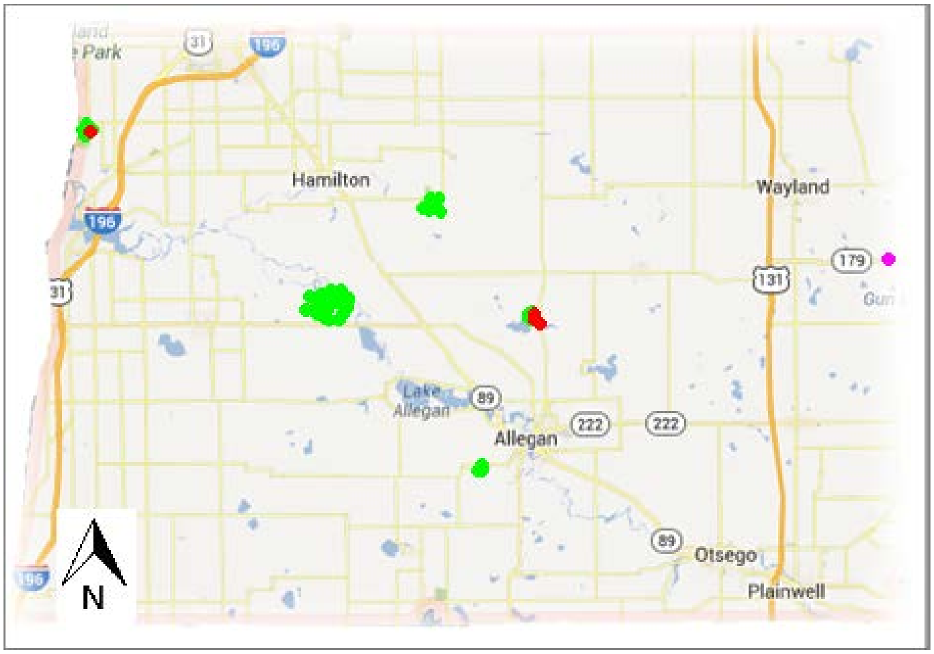


Figure 2: Map of latrine sites locations sampled in Barry County, Michigan. Green represents *Baylisascaris procyonis* (raccoon roundworm) negative sites. Red represents raccoon roundworm positive sites. Blue represents raccoon road kill without raccoon roundworm and purple represents raccoon road kill that contained raccoon roundworm.



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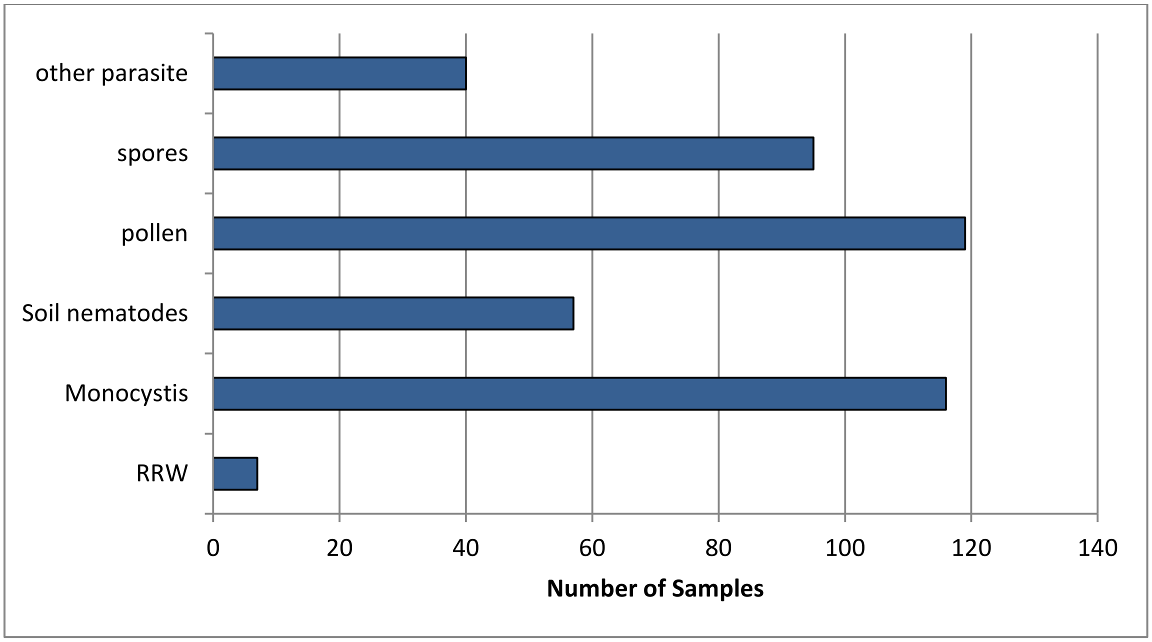

Figure 4: Artifacts found under the microscope in raccoon scat samples from Barry County, Michigan. (n=137)

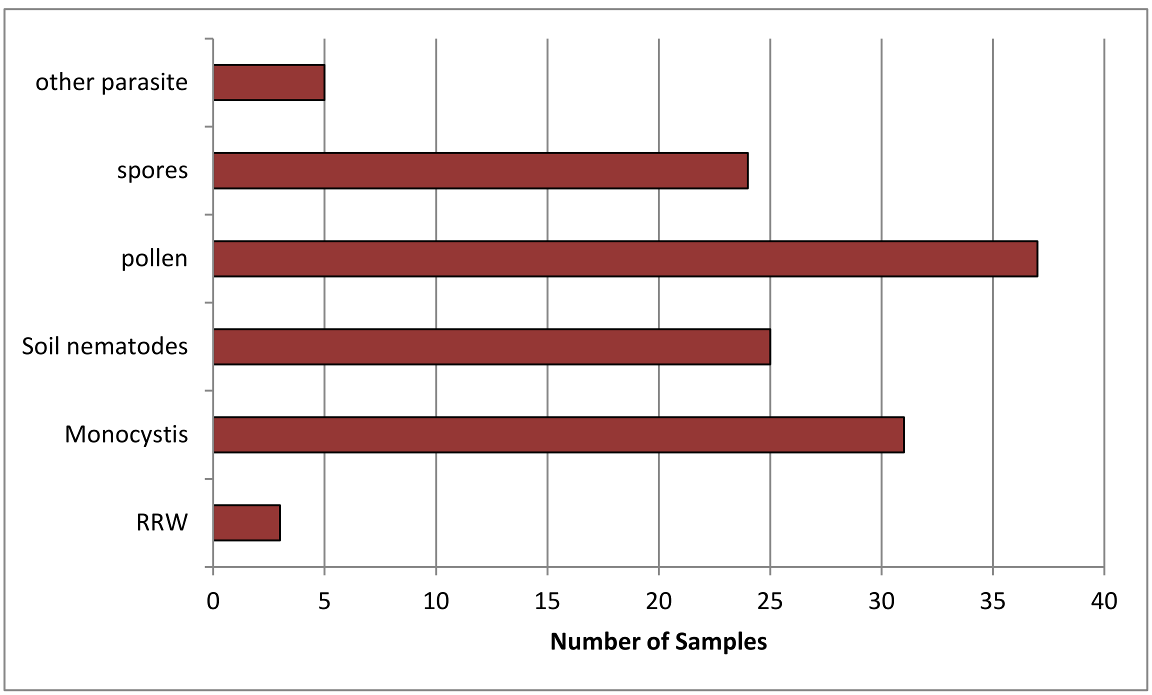

Figure 5: Artifacts found under the microscope in raccoon scat samples from Allegan County, Michigan. (n=54)

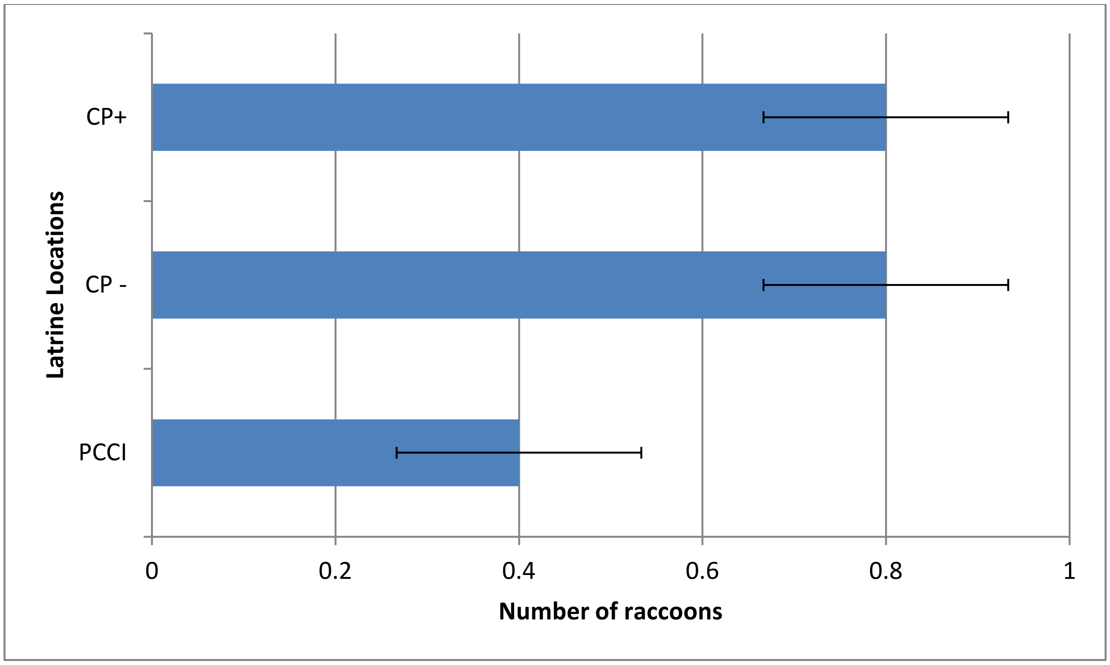

Figure 6: Raccoon visitations at latrine sites at Charlton Park and Pierce Cedar Creek Institute in Barry County, Michigan during a 5-day track plate study.

One-way ANOVA (df = 2, F = 0.36, P = 0.705)

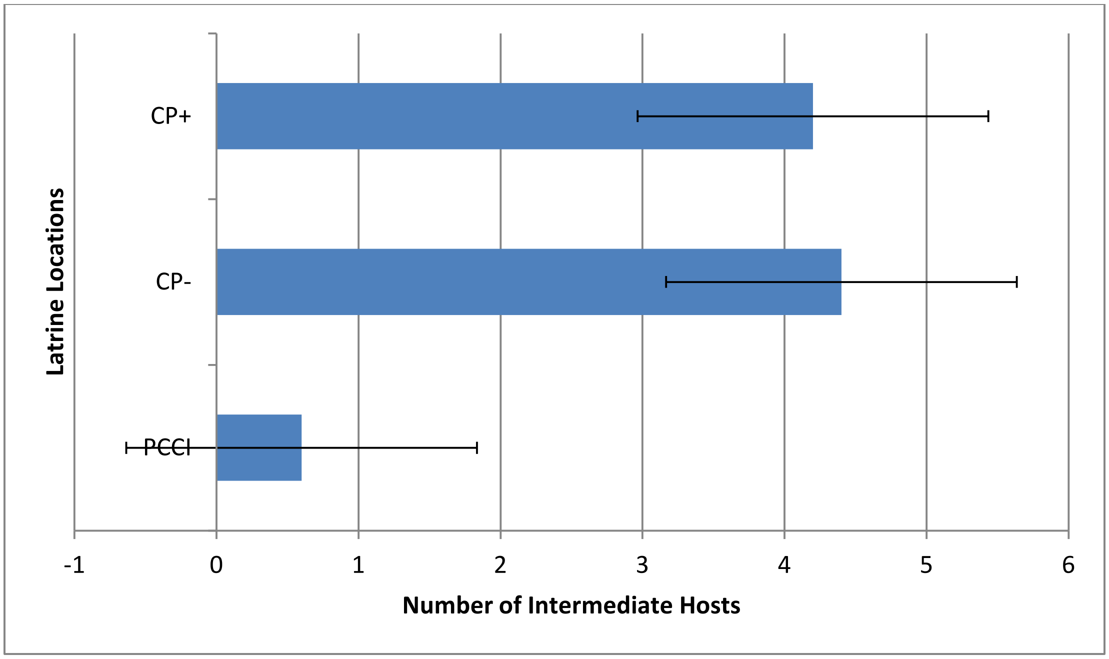

Figure 7: Intermediate host (*Tamias striatus*, *Sciurus carolinensis*, and *Peromyscus leucopus*) visitations at latrine sites at Charlton Park and Pierce Cedar Creek Institute in Barry County, Michigan during a 5-day track plate study. One-way ANOVA (df = 2, F=7.38, P = 0.008)

Discussion

We hypothesized that we would find a *Baylisascaris procyonis* infection rate similar to the 60 – 80% found in other related studies (Page *et al.* 1999; Page *et al.* 2008). From our results, we can conclude that raccoon roundworm is present in Barry and Allegan Counties, but not at the expected rates. Our study shows that the high prevalence rate as indicated before may not be as constant throughout the Midwest as previously thought. This leads us to believe that there may be spatial variation with the prevalence of *Baylisascaris procyonis.* We could clearly see spatial variation with a range of 0% up to 24% within our sites in Barry and Allegan Counties.

We did see a higher prevalence rate in our necropsied raccoons, an overall infection rate of 33%. We also found that there were more infected juvenile raccoons than adult raccoons and that there were more infected males than females. This coincides with previous studies (Roussere *et al.* 2008). Another observation was that raccoons that were necropsied later in the summer were more likely to be infected than raccoons that were necropsied earlier in the summer. This observation suggests that there may be a seasonal component to this disease. We also need to consider the possibility of our lower prevalence rates due to false negatives. If there is seasonality as well as a spatial aspect to this disease then scat samples collected could be from raccoons that have just become infected or have immature worms that may not be shedding eggs at that exact time of defecation. Even though these raccoons are infected, the eggs would not be seen in the fecal flotation process, leading to a false negative. We know that this may be a possibility because fecal samples were collected from all necropsied raccoons, and we found that even though some were positive for the adult *Baylisascaris procyonis* worms, there were no eggs found in the corresponding fecal floats.

The track plate results for raccoon visitations showed that there was no significant difference between the number of visitations to the positive, negative, and control sites; however, there were more visitations at Charlton Park than there were at PCCI. For intermediate hosts, there was also a difference in the number of visits to sites at Charlton Park and sites at PCCI. We hypothesized that positive sites would have higher intermediate host densities than negative sites as seen in previous research (Gompper and Wright 2005). Our results do not support this hypothesis of positive and negative sites being different, but it did show that there was variation between locations. Charlton Park had more intermediate host visits than PCCI and also had a higher prevalence rate of raccoon roundworm. This indicates that there may be a correlation between overall intermediate host densities and raccoon roundworm prevalence.

There are still many aspects of the *Baylisascaris procyonis* life cycle that are unclear. We suggest further research be carried out to look at the possibility of a seasonal component as well as the spatial variability of raccoon roundworm distribution. If the factors that are keeping raccoon roundworm prevalence low in certain areas can be determined, they could be used as a tool to keep the prevalence in other areas low as well. Further research will aid in getting a clear understanding of the effects this parasite causes as well as ways to prevent and lower its spread.

Acknowledgements

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