Prevalence and Distribution of *Baylisascarsis procyonis* in Raccoon Populations at Pierce Cedar Creek Institute in Barry County, MI

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Abstract

Baylisascaris procyonis is a species of ascarid worm that lives within the intestinal tract of the common raccoon (*Procyon lotor*). Eggs are expelled through defecation, and millions can accumulate at latrine sites habitually used by raccoons. Many animals, including humans, serve as intermediate hosts to the indirect development of the nematode. If ingestion of eggs occurs they can develop into ocular, visceral and neural migrans which leads to severe brain damage and if untreated, mortality. Our goal was to determine the overall infection rate and parasite load of the raccoon population at Pierce Cedar Creek Institute near Hastings, MI. We located raccoon latrine sites and fecal samples were collected and examined for presence of *B. procyonis* eggs. A total of 347 fecal samples were collected from 157 latrines. A 0% prevalence rate of *B. procyonis* was found, which meant a low environmental and public health safety risk at the time of our sampling. *Monocystis,* a psuedoparasite found in earthworms was found in 7% of our samples, although this parasite causes no harm to raccoons or humans.

Introduction

Baylisascaris procyonis, or raccoon roundworm, is a parasitic nematode found in the intestinal tract of the common raccoon (*Procyon lotor*). Raccoon roundworm is a known cause of neural, ocular, and visceral larval migrans in humans as well as a range of other intermediate hosts (Roussere *et al.* 2003; Gavin *et al.* 2005). Larval migrans coincide with an indirect path of development and are the cause of central nervous system diseases in intermediate hosts (Murray and Kazacos 2004), but has little or no impact on raccoons. Eggs are shed at high rates through the feces of their definitive host, up to 179,000 eggs/worm/day (Gavin *et al.* 2005). Raccoons use communal latrine sites which accumulate feces from several individuals (Kazacos 2001; Roussere *et al*. 2003; Page *et al.* 2009). This social behavior, paired with a high infection rate (up to: 86% in Illinois, 74% in Indiana, 25% in Ohio, and 58% in Michigan; Schultz 1962; Dubey 1982; Snyder and Fitzgerald 1987; Kazacos 2001), 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).

Baylisascaris procyonis has direct and indirect lifecycles (Roussere *et al.* 2003; Center for Disease Control) (Fig. 1.). Direct transmission to raccoons is age dependent. Juveniles are susceptible to direct infection and may become contaminated by direct ingestion of eggs (Roussere *et al.* 2003; Gompper and Wright 2005; Yeitz *et al.* 2009). This may occur during investigative behavior, scavenging for undigested seeds in infected raccoon feces, or communal grooming activities (Roussere *et al.* 2003). Adult raccoons appear to be resistant to direct infection and must ingest an intermediate host infected with *B. procyonis* (Roussere *et al.* 2003; Gompper and Wright 2005; Yeitz *et al.* 2009).

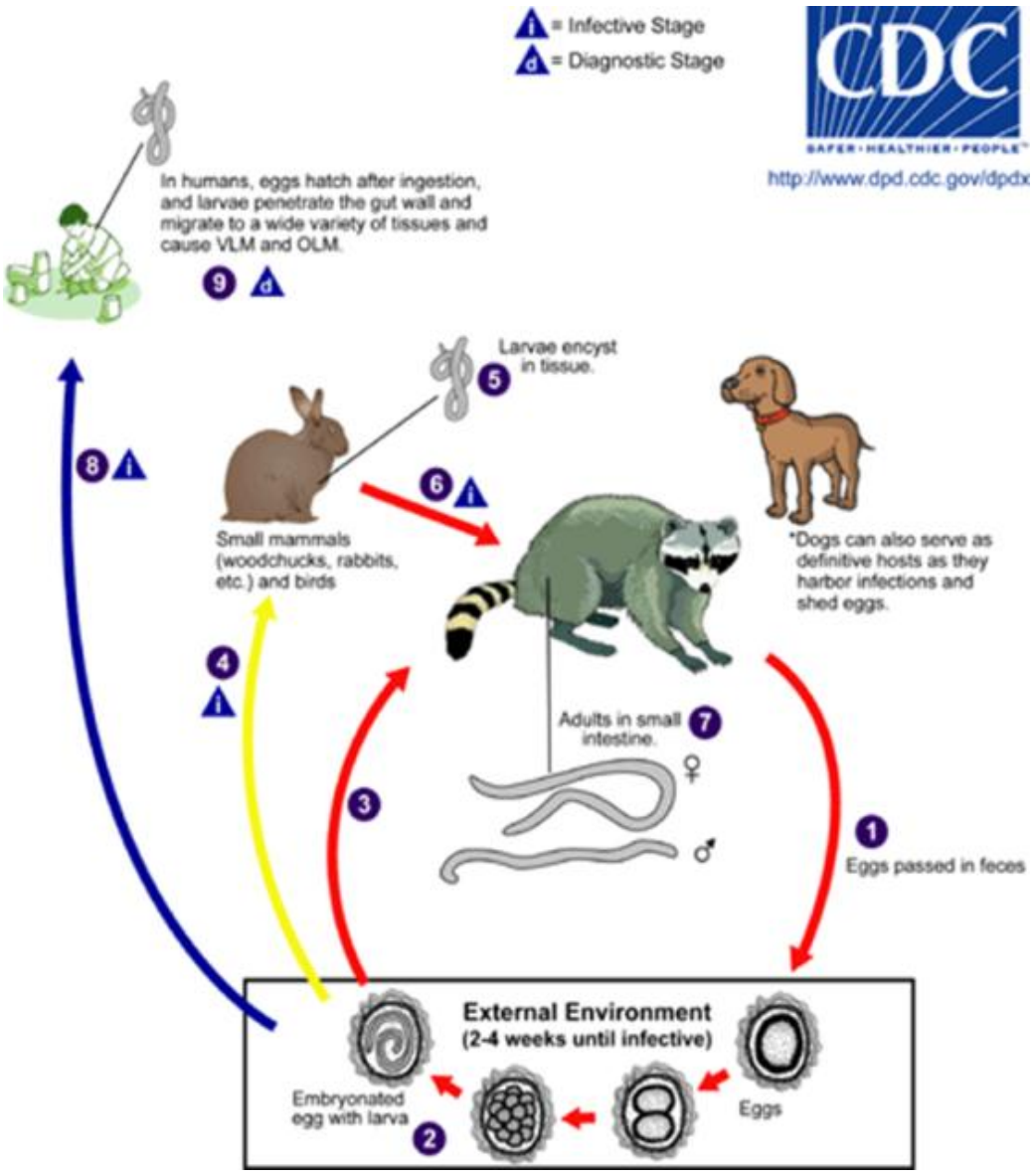

Figure 1. Direct and indirect life cycles of *Baylisascaris procyonis.* Source: Center of Disease Control.

A wide range of intermediate hosts (>90 species) scavenge at raccoon latrine sites in search of undigested seeds whereby accidental ingestion of infected eggs occurs (Page *et al.* 1999; Roussere *et al.* 2003; Yeitz *et al*. 2009). At Pierce Cedar Creek Institute (PCCI) common

intermediate hosts would likely include white-footed mice (*Peromyscus leucopus*), gray squirrels (*Sciurus carolinensis),* American Crows (*Corvus brachyrhynchos*), and American Robins (*Turdus migratorius*) (Evans 2002; Page *et al.* 2011). When intermediate host densities are low, raccoon infection rates are comparatively lower than in higher density areas (Gompper and Wright 2005). Although rare, cases of human infection are most common amongst children and individuals that exhibit behaviors such as pica or geophagia (Murray and Kazacos 2004). The severity of these cases is dependent on the number of eggs ingested, so may range from asymptomatic to death (Page *et al.* 2008; Sexsmith *et al.* 2009).

Raccoons are one of the most common members of the order Carnivora in North America (Gompper and Wright 2005). The species habituates readily to human activity and thrives in urban environments (Sexsmith *et al.* 2009). Raccoon latrine sites in close proximity to human development pose a public health risk. This is of concern in an area such as PCCI because of frequent trail use which puts humans in close proximity to latrine sites. *B. procyonis* eggs are extremely viable, resistant to desiccation and changes in pH and can remain in an environment for years (Shafir *et al.* 2011).Therefore *B. procyonis* eggs may be present without obvious signs of raccoon feces.

Acquiring information on the infection level of raccoon populations is the first step in creating a management plan for controlling raccoon roundworm. The desensitization of raccoons to human activity, potentially high infection rates, high viability of eggs and potentially fatal health hazards make identifying areas of *B. procyonis* presence important. Given that previous studies have found high infection rates (up to 82%) in the Midwest (Kazacos 2001), we hypothesized a high infection rate would also be found at PCCI in Southwest Michigan.

Methods and Procedures

We systematically searched for latrine sites on Pierce Cedar Creek's (PCCI) property near Hastings Michigan during April to May 2012. Latrines were located on fallen logs, tree bases, rocks and on ground floor. Latrines were flagged and GPS coordinates were recorded. Searching for latrines took place over a four-week period. We defined latrines as single or multiple scats separated by > 5 meters from other scats (e.g. fallen logs, tree bases, etc.). Only active latrine sites were sampled and fresh feces from these active sites were tested for presence of *B. procyonis* eggs. Once shed, eggs take approximately 2 to 4 weeks to become infective depending on environmental conditions, making fresh feces considerably less hazardous to handle (Murray and Kazacos 2004). Freshly collected scat was stored in Ziploc bags and frozen at -20 °C until tested. Freezing does not affect *B. procyonis* egg viability, so would not bias our ability to detect their presence (Smyser *et al.* 2010). Fecal flotation was performed to detect *B. procyonis* eggs and was carried out using a modified detergent wash flotation procedure with Sheather's sugar solution (specific gravity 1.25-1.27) and a standard Fecalyzer fecal float device (Page *et al.* 2009; Roussere *et al.* 2003; Sexsmith *et al*. 2009). Once fecal floatation was complete samples were analyzed using a light microscope at 100X for identification of *B. procyonis* eggs. Entire slides were examined for the eggs. All utensils and counters were wiped down with an all-purpose cleaning solution, which removes the eggs' adhesive coat and aids in surface removal, and then were heated to over 64°C which killed any potentially missed eggs (Sexsmith *et al.* 2009; Shafir *et al.* 2011). A blow torch was used to sterilize counter tops and utensils. Infection rates of local raccoon populations were estimated in two ways, as the

proportion of (1) fecal samples and (2) individuals found to be infectious from the sampled populations.

We also conducted a mark-recapture study of *P. lotor* to estimate how many raccoons visited latrine sites. Live traps (Tomahawk Live Traps LLC, Hazelhurst, WI) were deployed around each latrine for a two-week marking period. Six traps were utilized at each latrine. Traps were baited at night with sardines and checked in the early morning. Captured raccoons were moved into a squeeze cage to ear tag, after which they were released at the point of capture. Any feces in live traps were collected for examination. Following the two-week marking period, we used wildlife cameras (Scoutguard HD infrared DVR VGA 8GB cameras; HCO Outdoor Products, Norcross, GA) to monitor latrine sites for an additional two weeks. We used the resulting photographs to 'recapture' individuals, using the ear tag to identify marked individuals.

Images produced during the two-week recapture phase were scanned for potential intermediate hosts that were observed visiting or in proximity to raccoon latrine sites. Animals foraging or standing in scat were considered to be potential intermediate hosts. We also used wildlife cameras to document movement around live traps during the two-week marking period to determine how often we captured a raccoon that was in proximity to our live traps.

Results

We found 157 latrines on the PCCI property from which we collected 347 scat samples (Figure 2). The majority of latrines across the property were located either on the base of a tree or a fallen log (94%). Latrines on the south side of the property were mainly found on tree bases (89%). Latrines on the north side of the property were found to be a nearly equal mix of fallen logs and tree bases (Fallen log, 47%; Tree base, 46%). None of the 347 fecal samples tested positive for *B. procyonis* eggs, but 7% of the samples contained a psuedoparasite known as *Monocystis*.

During the live capture portion of our study 19 total raccoons were captured. The mean number of captured raccoons across all 6 latrines was found to be 3.17 ± 2.64 raccoons, with a mean of recapture events at 1.17 ± 1.60 raccoon. Only one latrine, JJJ, had no captures (Table 1). We captured images of seven raccoon visitations to latrine sites (Table 2). Latrine MMM had the highest number with two individuals sighted at the same time (Figure 3), while latrine R had two images of raccoons sighted at different times. Due to ear tags not being visible on the wildlife cameras it could not be determined if these were two separate individuals. All other latrines either only had one or no visitations.

We documented 32 total visitations of potential intermediate hosts (Table 3). Members of the family Sciuridae made up the largest number of visitations at 17 (53%) (Figure 4).

During the two-week marking period only 2 of 28 (7%) of photographed raccoons were captured (Table 4). Traps were observed for a one week period with wildlife cameras, and raccoons that were not trapped exhibited behaviors such as circling traps, partially entering traps, or wandering within the proximity of the traps.

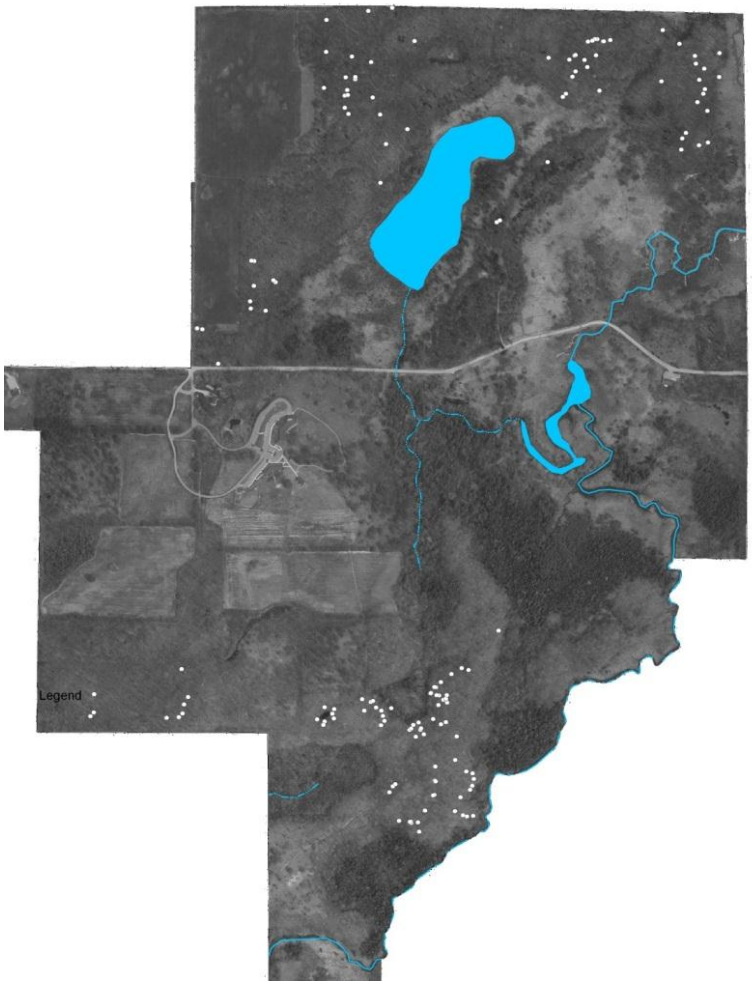

Figure 2. A map of Pierce Cedar Creek Institute near Hastings, Michigan showing the position of the 157 latrines (white dots) found on the property.

Table 1. Number of raccoons captured and recaptured over a two-week marking period at six latrine sites on Pierce Cedar Creek Institute near Hastings, MI.

Latrine I.D.	Number of Individual Raccoons	Recaptures
JJJ	0	0
MMM	2	1
R	7	4
KKK	1	0
Q	4	2
W	5	0

Table 2. Number of raccoon latrine visitations over a two-week camera monitoring period at six latrine sites on Pierce Cedar Creek Institute near Hastings, MI.

Latrine I.D.	Novel Events (Raccoon visitations)
JJJ	1
MMM	2
R	2
KKK	1
Q	1
W	0

Table 3. Number of intermediate host visitations over a two-week period field camera monitoring period at six latrines on Pierce Cedar Creek Institute near Hastings, MI.

Intermediate Host I.D.	Common Name	Visitation Frequency
Didelphis virginiana	**Virginia Opossum**	**2**
Sciurus carolinensis	**Eastern Gray Squirrel**	**10**
Sciurus niger	**Fox Squirrel**	**5**
Sciurus vulgaris	**Red Squirrel**	**1**
Tamias striatus	**Eastern Chipmunk**	**1**
Peromyscus leucopus	**White Footed Mouse**	**2**
Odocoileus virginianus	**White Tailed Deer**	**3**
Turdus migratorius	**American Robin**	**7**
Colaptes auratus	**Northern Flicker**	**1**
Picoides villosus	**Hairy Woodpecker**	**1**

Table 4. Number of raccoon visitations over a one-week trap monitoring period at three latrines on Pierce Cedar Creek Institute near Hastings, MI.

Latrine I.D.	Trap visitations (Captured)	Trap visitations (Uncaptured)
R	**1**	**3**
W	**0**	**11**
Q	**1**	**12**

Figure 3.
Two raccoons crossing a latrine site at midnight.

Figure 4.
An Eastern Gray Squirrel crossing a latrine site.

Discussion

We predicted a high prevalence rate for *B. procyonis* at Pierce Cedar Creek Institute, given the relatively high density of raccoons on their property. However, we documented a zero prevalence rate at PCCI. The absence of *B. procyonis* over a 268 ha sampling area, 157 latrine sites and 347 scat samples was uncommon relative to other recent studies in the Midwest region (Page *et al.* 1999; Page *et al.* 2008).

The *Monocystis* parasite was found in 7% of scat samples. *Monocystis* does not harm vertebrate animals, thus humans and raccoons would not be at risk (Sheridan 1986). When earthworms begin reproduction, the immature sporozoties, which can contain *Monocystis*, are in the soil. When their cocoon is broken *Monocystis* is released into the soil. As raccoons forage on the forest floor, soil and other decomposing debris are consumed and the raccoon ingests *Monocystis.* It then passes through its digestive system and is deposited in scat (Sheridan 1986).

We live trapped raccoons at six latrine sites to document how many raccoons visit individual latrine sites, something that has not been reported in the literature to the best of our knowledge. Nineteen raccoon individuals were captured over six different latrines, seven of which were recaptures at the same latrine site.

The wildlife cameras revealed seven raccoon visitations over a two-week monitoring period at six latrine sites. The number of raccoons utilizing a latrine site was found to range from 1 to 7 with a mean of 3.8 (Table 1). Unfortunately our method of marking raccoons with metal ear tags was not able to be seen on the wildlife cameras so we could not produce an estimate of how frequently individuals visited latrine sites.

With wildlife cameras we documented the Eastern Grey Squirrel, with 10 visitations, as the most frequent potential intermediate host at latrine sites. Intermediate hosts may forage in the scat for food and nutrients or may just walk through it and ingest the eggs as it cleans itself with its contaminated feet (Evans 2002; Page et al. 2011). Once the intermediate host has ingested eggs, the eggs can hatch in response to low pH in the stomach. The larvae burrow through the small intestine and then some larval migrans may infest neural tissue leading to neurological effects. As the infestation of *B. procyonis* takes over the brain, the animal moves more slowly and may exhibit rolling or circling behavior. This makes it easier for raccoons to consume the prey. Once a raccoon has ingested an infected intermediate host a similar process occurs, except that *B. procyonis* attach to the small intestine to grow and subsequently reproduce (Kazacos 2001).

Latrine trap visitations were significantly higher than raccoons that were actually captured. The scent of the sardines may have drawn *P. lotor* toward the traps. However, only two were actually captured from those visitations. This may be a result of them pulling the bait through the trap from the outside, grabbing the bait while inside and not setting off the trigger or avoiding the trap all together.

At the time of our sampling, PCCI property appears raccoon round worm free, however it could be present in the future which would require monitoring to assess. Given the relatively high density of raccoons on the PCCI property and the number of individuals intensively using this space it warrants future consideration. To help manage the transmission to humans, education about latrine sites and how infection is spread should be provided. When raccoons are in close proximity together the parasite can be spread more rapidly. By reducing accessible

garbage and other objects, the spread of this parasite can be minimized, and human exposure can be reduced.

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