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IMPLICATIONS OF RACCOON LATRINES IN THE EPIZOOTIOLOGY OF BAYLISASCARIASIS

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ABSTRACT: Raccoons (*Procyon lotor*) frequently establish defecation sites, termed latrines, on large logs, stumps, rocks, and other horizontally oriented structures/surfaces. These latrines are important foci of infective eggs of *Baylisascaris procyonis,* a nematode parasite of raccoons which is pathogenic to numerous species of mammals and birds. To examine the role of raccoon latrines in this animal-parasite interaction, we documented animal visitations to raccoon latrines in two large forested tracts and two woodlots in Indiana (USA) during 1994 and 1995. Species richness of vertebrate visitors did not differ between sites or years, but species composition differed by site and year. Fourteen mammal and 15 bird species were documented visiting raccoon latrines. Small granivorous mammals, including white-footed mice (*Peromyscus leucopus*), eastern chipmunks (*Tamias striatus*), and tree squirrels (*Sciurus carolinensis, S. niger, Tamiasciurus hudsonicus*) were the most common visitors to latrine sites. White-footed mice, chipmunks, white-breasted nuthatches (*Sitta carolinensis*), and opossums (*Didelphis virginiana*) were photographed foraging on undigested seeds in raccoon feces. Active foraging at latrines also was shown experimentally; seeds embedded in raccoon feces were removed at a greater rate at latrine sites than at nonlatrines. We conclude that raccoon latrines are visited routinely by a variety of vertebrates, especially small granivorous rodents and birds which forage for seeds in raccoon feces, and that raccoon latrines are probable sites of transmission of *B. procyonis* to susceptible mammals and birds.

Key words: Ascarid, *Baylisascaris procyonis,* granivore, nematode parasite, *Procyon lotor,* raccoon latrine.

INTRODUCTION

Free-ranging raccoons (*Procyon lotor*) typically defecate in preferred sites, termed latrines (Giles, 1939; Yeager and Rennels, 1943; Stains, 1956). In forested areas, raccoon latrines are found most often at the base of trees, in raised crotches of trees, and on large logs, stumps, rocks, tree limbs, and other horizontally oriented structures/surfaces (Yeager and Rennels, 1943; Stains, 1956; Cooney, 1989; Kazacos and Boyce, 1989; Page et al., 1998). Any given raccoon latrine is often visited by many individual raccoons (Page, 1998), and in areas where raccoon populations are high, substantial quantities of raccoon feces accumulate at raccoon latrines.

Raccoons are the definitive hosts of *Baylisascaris procyonis,* an intestinal ascarid nematode which commonly produces larval migration-related disease in other animals. Prevalence of infection in raccoons may be high. For example, in the midwestern and northeastern United States, *B. procyonis* has been documented in 68 to 82% of raccoons (Kazacos and Boyce, 1989). Numerous small vertebrates are potential intermediate hosts of *B. procyonis* and become infected by accidentally ingesting infective eggs from the environment (Sheppard and Kazacos, 1997). *Baylisascaris procyonis* is a well-known pathogen for such mammals and birds, and could be an important morbidity and mortality factor in individuals and populations sharing or frequenting the habitat of infected raccoons. In intermediate hosts, *B. procyonis* larvae undergo aggressive somatic migration, often entering and damaging the central nervous system (CNS) and producing clinical CNS disease (Kazacos and Boyce, 1989). *Baylisascaris procyonis* is the most commonly recognized cause of clinical larva migrans in an-

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imals, having produced fatal or severe neurologic disease in over 50 species of mammals and birds (Sheppard, 1995; Kazacos, 1997).

Female *B. procyonis* are prolific egg producers, and infected raccoons shed an average of 20,000 to 26,000 eggs/g feces, ranging as high as 256,700 eggs/g (Kazacos, 1983; Snyder and Fitzgerald, 1987). Large numbers of *B. procyonis* eggs accumulate in the feces and soil at raccoon latrines, and these eggs can remain viable and infective in the environment for years (Kazacos, 1983; Kazacos and Boyce, 1989). Raccoon latrines have become increasingly suspected as important areas of animal–parasite interaction involving transmission of *B. procyonis* to various mammals and birds (Kazacos and Boyce, 1989; Sheppard and Kazacos, 1997).

Previous studies have indicated that raccoon latrines are foci of *B. procyonis* eggs, and probably serve as long-term sources of infection for susceptible animals (Jacobson et al., 1982; Cooney, 1989). However, important questions remain concerning the dynamics of *B. procyonis* transmission to intermediate hosts and the effects of the parasite on intermediate host populations (Sheppard and Kazacos, 1997). We suspect that species utilizing habitats associated with raccoon latrines and/or having behaviors that bring them into contact with these areas would be at increased risk of infection with this parasite. Although raccoon latrines probably play an important role in the transmission dynamics of *B. procyonis,* the extent of animal-latrine and animal-parasite interaction occurring at latrines has not been documented.

Intermediate hosts could become infected by accidentally ingesting *B. procyonis* eggs while investigating areas associated with raccoon latrines, by foraging on undigested seeds and other materials in dried raccoon feces at latrines, or indirectly by grooming themselves after becoming contaminated at a latrine (Kazacos and Boyce, 1989; Sheppard and Kazacos, 1997). Since raccoon latrines contain an abundance of undigested seeds, corn and other potential food material, we felt that foraging by potential intermediate hosts would be an important risk factor for acquiring *B. procyonis* infection at these sites.

Because of the pathogenicity of *B. procyonis* for a variety of small vertebrates, it is important to know which vertebrate species engage in behaviors that would increase their risk of contacting infective eggs of this parasite. Our objectives in these studies were to determine the extent of visitation by vertebrates to raccoon latrines and to clarify the potential role of foraging among feces at latrines as a means of transmission of *B. procyonis* to small vertebrates. We attempted to determine if species differences exist in the frequency of visitation to raccoon latrines, to determine relative risk of infection with *B. procyonis* due to differences in visitation frequency, and to document whether foraging occurs at latrines, which would increase the potential for ingestion of infective eggs. We tested the hypothesis that granivorous species would be common visitors to latrines, frequenting these sites in order to forage for seeds or other food items present in raccoon feces.

MATERIALS AND METHODS

We examined vertebrate visitation to raccoon latrines at two large forested tracts of >1,000 ha (40°27′30″N, 87°05′30″W; 40°26′30″N, 87°01′30″W) and two small farmland woodlots (8.2 ha and 0.6 ha; 40°30′30″N, 87°01′30″W and 40°26′30″N, 87°10′30″W, respectively) in Tippecanoe and Warren counties (Indiana, USA). Raccoon latrines were located by walking line transects spaced at 15-m intervals. The entire area of each small woodlot was searched, while in the two forested sites, searches were limited to 4-ha grids.

Seasonal changes in the seed content of raccoon feces have no effect on the types of species visiting latrines (Page, 1998). Therefore, the timing of this study (September to November) corresponded with a natural increase in the seed content of raccoon feces rather than shifts in seed type. This increase in seed content also corresponds with a seasonal increase in the patency of *B. procyonis* infections in rac-

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coons (Kidder et al., 1989). Trailmaster™ 1500 camera systems (Lenexa, Kansas, USA) were used to monitor animal visitation activity at 14 latrines (7 woodlot and 7 forest) in 1994, and at eight latrines (3 woodlot and 5 forest) in 1995. Camera systems were rotated among the study sites from September to November of each year. The infrared trip beam of each unit was aligned over the active portion of the latrine approximately 0.5 cm above the surface. The interrupt pulse for the receivers was set so that a 0.10 second break (pulse = 2) in the infrared beam would register an event. This setting was sensitive enough to detect mouse-size vertebrates. Counters recorded the time and date of each break in the infrared beam. The cameras also were triggered by breaks in the beam; however, cameras were set for a 15-min delay following each photograph to increase the likelihood that film would last for 24-hr periods. Kodak Royal Gold™ 1,000 speed film was used in each of the cameras.

Statistical analyses were performed using SAS (SAS Institute, Inc, 1994, SAS/STAT guide for personal computers, version 6.10 edition, SAS Institute, Inc., Cary, North Carolina). Compared variables were determined to be significantly different at $P \leq 0.05$.

We tested differences in visitation frequencies between years using ANOVA. Because there were no significant differences, we pooled years for subsequent analysis. An analysis of cross-classified categorical data was used to determine whether there were differences in visitation frequency between animal species, or sites. Visitation data were analyzed using log-linear models, with species, sites, and years as main effects. Counter and photographic data were used to document all visitations for 5 day trials conducted at each latrine in both years. Photographs were used to document which species visited latrines, and were compared to counter data to develop visitation profiles for each species.

Logistic regression (SAS Institute, Inc.) was used to develop models for predicting the probability of occurrence at a latrine for documented species as a function of Julian date, hour, and study site. These models were then used to predict which species were represented by counter data lacking photographs (18% of all event data). An event without a photograph was assigned to the species with the highest predicted probability of occurrence.

To determine whether vertebrates were actively foraging at raccoon latrines, experiments were conducted in which corn-laden raccoon feces were placed at latrines and similar non-latrine sites. Raccoon feces were collected from a captive individual that had been dewormed previously using pyrantel pamoate. Twenty corn fragments were embedded in fecal piles (~40 g) to simulate feces found at latrines following the harvest of corn in fields adjacent to our study sites. Corn-laden feces were placed at established latrines ($n = 4$ in both 1994 and 1995) and at other locations with appropriate horizontal characteristics (Page et al., 1998) but no visible raccoon feces (nonlatrines) ($n = 4$ in 1994 and $n = 3$ in 1995). Animal activity was monitored and compared by chi-square analysis (Zar, 1984) for 3 day trials during January, at the end of which the remaining feces were collected and remaining corn fragments were counted. A binomial test with correction for continuity was used to compare seed removal from feces between latrines and nonlatrines, and between years (Zar, 1984).

RESULTS

During the 2 yr study, 252 animal visits to raccoon latrines were documented during 110 camera nights. The total number of visits to latrines remained constant across each 5-day trial. However, differences were noted in number of visits and types of species between sites. Fourteen mammal and 15 bird species were photographically documented visiting latrine sites (Table 1). In addition to raccoons, small granivorous mammals such as white-footed mice (*Peromyscus leucopus*), eastern chipmunks (*Tamias striatus*), and tree squirrels (*Sciurus carolinensis*, *S. niger*, *Tamiasciurus hudsonicus*) were the most frequent visitors to latrines (Table 2).

Visits by vertebrate species differed across sites ($\chi^2 = 18.67$, $P = 0.002$), the types of species represented by the visitation data differed between forests and woodlots (Table 2), and numbers of visits of some species differed between forests and woodlots. Raccoons ($Z_{94,95} = 3.64$, $P < 0.05$) and chipmunks ($Z_{95} = 12.88$, $P < 0.05$) visited latrines at forested sites more frequently than at woodlot sites. In contrast, white-footed mice visited latrines at woodlots more frequently than at forest sites ($Z_{94} = 3.04$, $P < 0.05$). The visitation frequencies of birds and tree squirrels were too low to include in the statistical analysis.

Active foraging for undigested seeds in

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TABLE 1. Species of mammals and birds visiting raccoon latrine sites in west-central Indiana, as documented photographically.

Species	Visitation trials		Foraging trials	
	Woodlot	Forest	Woodlot	Forest
White-footed mouse (*Peromyscus leucopus*)	X	X	X	X
Eastern Chipmunk (*Tamias striatus*)	X	X	X	X
Fox squirrel (*Sciurus niger*)	X	X	X	X
Gray squirrel (*Sciurus carolinensis*)		X		
Red squirrel (*Tamiasciurus hudsonicus*)	X	X		
Southern flying squirrel (*Glaucomys volans*)		X		
Woodchuck (*Marmota monax*)[a]				X
Eastern cottontail (*Sylvilagus floridanus*)	X			
Virginia opossum (*Didelphis virginiana*)	X	X	X	X
Raccoon (*Procyon lotor*)	X	X	X	X
Striped skunk (*Mephitis mephitis*)			X	
Long-tailed weasel (*Mustela frenata*)		X		
Red fox (*Vulpes vulpes*)[a]			X	
Domestic cat (*Felis domesticus*)	X	X		
Mourning Dove (*Zenaida macroura*)[a]	X			
White-breasted Nuthatch (*Sitta carolinensis*)			X	
Brown Thrasher (*Toxostoma rufum*)	X			
Blue-Jay (*Cyanocitta cristata*)	X			
Hermit Thrush (*Catharus guttatus*)	X	X		
Swainson's Thrush (*Catharus ustulatus*)		X		
Wood Thrush (*Hylocichla mustelina*)		X		
Ovenbird (*Seiurus aurocapillus*)	X	X		
Northern Junco (*Junco hyemalis*)			X	X
Downy Woodpecker (*Picoides pubescens*)			X	
House Wren (*Troglodytes aedon*)		X		
Black-capped Chickadee (*Parus atricapillus*)	X			
Cardinal (*Cardinalis cardinalis*)	X			
Carolina Wren (*Thryothorus ludovicianus*)	X	X		
Eastern Screech Owl (*Otus asio*)[a]	X			

[a] Documented, but not included in analysis.

raccoon feces was documented at established latrines and at nonlatrine sites. Corn was removed from experimental fecal piles placed at both latrine and nonlatrine locations. However, the mean number (± SE) of seeds removed from feces at latrine sites (12.75 ± 3.22) was significantly greater ($Z = 6.96$, $P < 0.05$) than the number removed at nonlatrine sites (4.43 ± 2.81). We photographically documented active foraging at established latrines by a white-breasted nuthatch (*Sitta carolinensis*), and recorded eight instances of mice and 10 of chipmunks either standing on fecal piles, positioned with their mouths over seed-laden feces, or having seed in their mouths. We also photographed an opossum (*Didelphis virginiana*) with a large piece of raccoon feces in its mouth. Numerous other photographs were taken of small granivorous mammals inspecting the seeds in feces at latrines.

DISCUSSION

Our results provide evidence that raccoon latrines are visited routinely by a variety of small vertebrates. Visitation was confirmed at all latrines monitored during the study. Our documentation of visitation to latrines by numerous species of mammals and birds (Table 1) is consistent with the hypothesis that most transmission of *Baylisascaris procyonis* to intermediate hosts in nature is through ingestion of infective eggs present at raccoon latrines. These results correlate well with those of

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TABLE 2. Number of visits by selected vertebrates to raccoon latrine sites in west-central Indiana. Numbers in parentheses represent percent of total visits at a site.

Species	W[a]	F[b]	Total
Raccoons	10 (10)	36 (23)	46 (18)
Opossums	6 (6)	4 (3)	10 (4)
White-footed mice	49 (49)	26 (17)	75 (30)
Eastern chipmunks	6 (6)	49 (32)	55 (22)
Fox, gray, and red squirrels	14 (14)	22 (15)	36 (14)
Birds	11 (11)	9 (6)	20 (8)
Other	4 (4)	6 (4)	10 (4)

[a] W = Woodlot
[b] F = Forest

Cooney (1989), who found that *B. procyonis* eggs were rarely recovered (once) from randomly obtained soil samples in a heavily wooded urban park, but were instead distributed nonrandomly, i.e., were associated with raccoon latrines. In fact, *B. procyonis* eggs were found in 18% (Page et al., 1998) to 20% (Cooney, 1989) of soil and fecal samples taken from known raccoon latrines. Active raccoon latrines are characterized by a constant addition of fresh feces to older decomposing fecal piles. *Baylisascaris procyonis* eggs found in feces at raccoon latrines are at varyious stages of development, and reach infectivity relatively quickly or slowly depending on ambient temperature. At 22 to 25 C, *B. procyonis* eggs became infective in 11–14 days (Sakla et al., 1989), whereas under natural conditions development may take several weeks to months (Kazacos, 1983; Kazacos and Boyce, 1989). Eggs found in soil associated with raccoon latrines originate from older broken-down fecal piles; accumulating over a period of time, therefore, they are commonly larvated and potentially infective to intermediate hosts. *Baylisascaris procyonis* eggs are resistant to environmental conditions and may remain infective in soil and feces at non-active raccoon latrines for years (Kazacos and Boyce, 1989). Consequently, the long-term accumulation of raccoon feces at latrines combined with the longevity of infective *B. procyonis* eggs results in an increased potential for transmission to occur at latrines.

Because intermediate hosts become infected with *B. procyonis* by ingesting infective eggs, it is important to know the frequency with which these potential hosts contact latrine foci which contain the eggs. Small granivorous mammals such as white-footed mice, eastern chipmunks, and tree squirrels were the most frequent visitors to raccoon latrines, and it is likely that these species are the most common intermediate hosts for *B. procyonis.* Documentation of larval *B. procyonis* infections in granivorous species living in areas with abundant raccoon latrines provides further support for the thesis of transmission via foraging at raccoon latrines. Tiner (1953, 1954) documented natural infections with *B. procyonis* larvae in 14 of 16 white-footed mice and eight of 12 fox squirrels (*Sciurus niger*) in Illinois woodlots containing abundant raccoons, and estimated that 5% of natural mortality of white-footed mice was due to *B. procyonis* infection. *Baylisascaris procyonis* larvae also were frequently found in *P. leucopus,* with and without CNS disease, taken from wooded areas in west-central Indiana (C. H. Sheppard, pers. comm.). Recently, Page (1998) identified *B. procyonis* infections in 111 of 487 *P. leucopus* trapped from habitats in Indiana with frequently occurring raccoon latrines. *Baylisascaris procyonis* infection was also documented as a common cause of clinical CNS disease in gray squirrels (*Sciurus carolinensis*) in Washington (Tseng, 1997). Natural infections with *B. procyonis* larvae also have been documented in six *S. carolinensis,* two *S. niger,* and one *T. striatus* (K. R. Kazacos, unpubl. data) from Indiana. Based on the results of the present study, most of these animals probably became infected by foraging at raccoon latrines.

A wide variety of birds also visited raccoon latrines (Table 1), but visits by birds (8% of total) were much less frequent than visits by granivorous rodents (66% of to-

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tal). Although it would appear that, based on their lower frequency of visitation, birds would have a lower risk of *B. procyonis* infection, naturally occurring clinical CNS disease due to *B. procyonis* has been documented in over 20 species of birds, including small free-ranging songbirds, such as American robins (*Turdus migratorius*) and blue jays (*Cyanocitta cristata*), and larger birds, such as mourning doves (*Zenaida marcroura*) (Evans and Tangredi, 1985). Although direct evidence of foraging by birds at latrines is limited, the majority of bird species observed at latrines in this study were granivores or omnivores (67% of total), which could have been foraging while at the latrines.

In conjunction with this evidence of granivore activity at raccoon latrines, we also gathered experimental data showing active foraging for undigested seeds in raccoon feces. Seeds (corn) were removed at a greater rate from raccoon feces at established latrines than from feces at nonlatrines, indicating that granivores are familiar with latrines as sources of food. In fact, photographic evidence of mice carrying feces by mouth suggests that white-footed mice may cache feces from latrines. Caching behavior would be an important link to *B. procyonis* infection, since infective eggs would also be stored. If granivores recognize latrines as food sources and make repeated visits to these sites (Cooney, 1989; Page et al., 1998), their risk of contracting *B. procyonis* is subsequently increased. Since infective *B. procyonis* eggs are concentrated at these sites, it is likely that eggs would accidentally be ingested by granivores removing seeds directly from raccoon feces or from contaminated bark, soil, or other substrates at latrines.

Our results also show that in an agricultural landscape, raccoon latrines are utilized by different types of species in large versus small forest patches. Nupp (1997) documented differences in types of small mammals in forest fragments of differing size and isolation. Species at a higher risk of infection with *B. procyonis* in small habitat patches appear to differ from those at higher risk in larger forested tracts, presumably as a function of fragmentation-induced differences in species composition. For example, white-footed mice were the most frequent visitors to raccoon latrines in small woodlots, whereas chipmunks were more likely to visit latrines in larger forests. Not surprisingly, white-footed mice occur in greater densities in small woodlots, which lack other granivorous mammals (Nupp and Swihart, 1996; Nupp, 1997). Therefore, the identity of intermediate hosts important in the transmission of *B. procyonis* back to raccoons might differ predictably across landscapes as a function of the degree of agriculturally induced fragmentation of forests.

Finally, our results of significant animal–latrine interaction indicate that the prevention and control of *B. procyonis* larva migrans in wild animals may be difficult to achieve without the direct management of raccoons in an area. Because raccoon latrine sites are so attractive to other animal species, the only way to decrease transmission of *B. procyonis* to other animals in an area would be to substantially decrease the prevalence of the parasite in the raccoon population, decrease the occurrence of raccoon latrines, or both. Decreasing the prevalence of *B. procyonis* in free-ranging raccoons, such as by bait deworming, is theoretically possible; however, the logistics and effectiveness of such an approach are unknown (Kazacos et al., 1991). Alternatively, depopulation and removal of raccoons from an area, combined with decontamination of established latrines, would immediately reduce new and existing sites of contamination. Decontamination of latrines would best be accomplished using heat, such as from a portable propane flame gun (Kazacos and Boyce, 1989). Based on our results, it is apparent that without some management of the raccoon population in an area, it would be difficult or impossible to reduce or elimi-

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nate *B. procyonis* transmission to other sympatric species.

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