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Raccoon Latrine Structure and Its Potential Role in Transmission of Baylisascaris procyonis to Vertebrates

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ABSTRACT.—*Baylisascaris procyonis*, the common large roundworm of raccoons (*Procyon lotor*), causes clinical neurologic disease in many species of mammals and birds. Infective eggs of *B. procyonis* are present at raccoon latrine sites, and these sites may be important in the transmission of this parasite to syntopic small vertebrates in forested areas. We located raccoon latrines in forested sites in Indiana, sampled soil and fecal material from these locations, and examined these samples for the presence of *Baylisascaris procyonis* eggs. We also quantified the structural characteristics of raccoon latrines in wooded areas, compared their characteristics with randomly located sites, and classified sites based on structural features using stepwise discriminant function analysis. *B. procyonis* eggs were present at 14% of the raccoon latrines sampled. Latrine sites differed from randomly located sites and exhibited characteristics generally associated with treefall gaps. Most latrines were located either on logs (49%) or at the base of large trees (37%). Structural features surrounding latrines often are important travel routes or foraging areas for various small vertebrates. The visitation of mammals and birds to sites exhibiting these structural features may result in infection with *B. procyonis*. In this way, *Baylisascaris procyonis* could have long-term impacts on populations of native mammals and birds.

INTRODUCTION

Descriptions of habitats used by raccoons (*Procyon lotor*) commonly refer to the presence of tree cavities, downed logs and rocky crevices (Giles, 1942; Stuewer, 1943; Berner and Gysel, 1967; Lehman, 1977, 1984; Kennedy *et al.*, 1991). Snags and logs are important components of raccoon habitat that provide den and refuge sites, travel routes, and sources of invertebrates suitable for food (Kennedy *et al.*, 1991). These features also are associated with accumulations of raccoon feces resulting from habitual defecation at sites called latrines (Stains, 1956; Kennedy *et al.*, 1991). Giles (1939), Stains (1956) and Yeager and Rennels (1943) described raccoon latrines as single or accumulated piles of scats on logs or at the base of trees. Latrines also have been observed occurring on large rocks, rock ledges, on the top of bluffs, near den entrances, on debris piles, on stumps, and in the crotches of large trees (Giles, 1942; Tevis, 1947; Yeager and Rennels, 1943; Tester, 1953; MacLintock, 1981; Cooney, 1989).

Our interest in raccoon latrines stems from the important role they appear to play in the transmission of *Baylisascaris procyonis,* the common large roundworm of raccoons, to small mammals and birds (Kazacos and Boyce, 1989; Sheppard and Kazacos, 1997). Young raccoons and a variety of intermediate host species become infected with *B. procyonis* through contact with larvated *B. procyonis* eggs which accumulate at raccoon latrines (Kazacos, 1983a; Kazacos and Boyce, 1989). Prevalence of *B. procyonis* in raccoons in the midwestern and northeastern United States can be high (68–82%), leading to significant environmental contamination with eggs(Kazacos and Boyce, 1989). Adult female *B. procyonis* are prolific egg producers (Kazacos, 1982), and the average infected raccoon sheds about 20,000 eggs per gram of feces (Kazacos, 1983a). *Baylisascaris* eggs passed in raccoon feces embryonate to the infective larval stage in about 3–4 wk and can remain viable in the environment for at least 5–6 yr (Kazacos, 1983a; Kazacos and Boyce, 1989). Raccoon latrines thus become important foci of infective eggs (Jacobson *et al.*, 1982; Cooney, 1989) and serve as long-term sources of infection for animals (Kazacos and Boyce, 1989; Sheppard and Kazacos, 1997). Intermediate hosts of *B. procyonis* become infected by accidentally ingesting infective eggs, presumably as a result of foraging at raccoon latrine sites or by grooming contaminated fur or feathers after traveling across a latrine (Sheppard and Kazacos, 1997).

Baylisascaris procyonis is remarkably nonspecific with regard to infection of potential intermediate hosts. Over 70 species of mammals and birds have been found to be susceptible to infection, including >55 species which have been naturally infected in the wild or in captivity (Kazacos, 1983a; Kazacos and Boyce, 1989; Sheppard, 1995). Infective eggs of *B. procyonis* hatch after ingestion by the intermediate host, and the newly hatched larvae penetrate the small intestine and migrate to various somatic tissues and visceral organs where they become encapsulated (Sprent, 1952, 1955; Tiner, 1953a,b; Sheppard, 1995). In fact, *B. procyonis* is considered to be the most common cause of clinical larva migrans in animals, in which it usually results in progressive clinical central nervous system (CNS) disease (Sheppard and Kazacos, 1997; Kazacos, 1997). Clinical signs of CNS include nervousness, lack of coordination, head tilt, ataxia, circling, recumbency and death (Tiner, 1953a,b; Kazacos and Boyce, 1989; Sheppard and Kazacos, 1997). *Baylisascaris procyonis* is also an important zoonotic parasite, and has caused fatal or severe CNS disease and ocular disease in humans (Huff *et al.*, 1984; Fox *et al.*, 1985; Goldberg *et al.*, 1993; Cunningham *et al.*, 1994). The nonspecificity for intermediate hosts and pathogenicity of *B. procyonis* may have a negative impact on populations of small vertebrates. For instance, it has been implicated as an important mortality factor for populations of white-footed mice (*Peromyscus leucopus*) in woodlots in Illinois (Tiner, 1954) and more recently as the cause of decline of populations of the Allegheny woodrats (*Neotoma magister*) in New York (MacGowan 1993).

Because raccoon latrines are possible foci of infection to intermediate hosts, a quantitative characterization of areas containing latrines could prove useful in predicting high-risk areas for transmission and in identifying those vertebrate species that are most likely to use such sites and thus become infected. Our objectives were to (1) determine the extent of *Baylisascaris procyonis* contamination at raccoon latrines, (2) quantify the structural characteristics of latrines and surrounding sites, and (3) determine whether characteristics of latrine sites differed from random locations.

METHODS

We surveyed four forested locations in Tippecanoe County, Indiana, using line transects at 15-m spacing to locate latrines. Latrines were identified based on the presence of raccoon fecal piles (Yeager and Rennels, 1943). Structural features associated with latrines were examined in one large forest (1354 ha) and in one small woodlot (8.2 ha) during April and May 1995. Structural features were measured at 45 latrines in the small woodlot and 37 in the forest. Twenty-five random sites were established at each study site. These sites were determined by randomly choosing a grid line, distance (0–195 m) along the grid line, direction perpendicular to the grid line, and distance (0–7.5 m) from the grid line within the 4-ha grids. Structural characteristics recorded for each latrine and random site included percent of ground covered by woody debris within a 5-m radius of the site, number of understory trees (≤10 cm dbh) within a 3-m radius of the site, number of overstory trees (>10 cm dbh) within a 5-m radius of the site, and the minimum distance of each site from the edge of the forest or woodlot. Woody stem density was estimated by counting the number of stems intercepting the line of sight to a 3-m cover board placed 5 m away from the center of the site at each of the four cardinal directions. Stem density was tabulated for the following vertical strata: 0–1 m, 1–2 m, 2–3 m aboveground. We also recorded the type of substrate (*i.e.*, fallen log, base of tree, stump, fork of tree, farm equipment, miscellaneous) on which latrines were located.

To predict structural features associated with raccoon latrines, latrine and random sites were classified using stepwise discriminant analysis. Original measurements of habitat variables were included in the analysis. A dummy variable representing substrate type also was included, where a value of 1 was assigned to any latrine occurring on a log, a stump, or at the base of a tree, and a value of 0 for other substrate types. To avoid subjectivity associated with assignment of substrate types to random locations, we did not record the substrate type occurring at the center of a random site. It is difficult to choose a random point on a line with any accuracy (Rudran and Foster, 1996); therefore, assignment of substrate type was achieved following collection of habitat data by generating a uniform random number between 0 and 100. If the random number was less than our estimate of the percent of a random site covered by woody debris and total basal area of standing trees, then the dummy variable for substrate type was assigned a value of 1. Otherwise, a value of 0 was assigned.

Soil and fecal material were sampled from latrines located for purposes of quantifying structural features and from latrines located at two other sites, a large (>1300 ha) forest in Tippecanoe County and a small (0.6 ha) woodlot in Warren County. For each latrine, samples were composites of all fecal piles present and soil from beneath feces. Availability of feces varied among latrines and resulted in variation in sample size, but all samples were ≥3 g. Presence of *Baylisascaris procyonis* eggs in soil and fecal material was determined using centrifugal flotation in Sheather's sugar solution, specific gravity 1.25. Soil samples were washed three times before flotation as described by Kazacos (1983b). Slides were then examined microscopically for the presence or absence of *B. procyonis* eggs.

RESULTS

Baylisascaris procyonis eggs were present at all four forested sites, and of the 137 latrines sampled, 14% had eggs present. At the time of the initial survey, latrines were active and fresh raccoon feces were present. However, at the time of the soil and fecal sampling not all latrines remained active, and fresh feces often were not present. Of the latrines where *B. procyonis* eggs were present, 68% were not active at the time of sampling.

Latrines were most commonly located on logs (49%) or at the base of trees (37%), with the remainder located on stumps (7%), in forks of trees (2%), farm equipment (1%), and on other substrates (4%).

Based on substrate type and distance to edge; the discriminant analysis correctly classified 127 of 132 of the locations (96.2%). Five of 82 latrines and none of 50 random locations were misclassified. Although distance to edge (m) was statistically significant ($F = 5.16$, $P = 0.025$, $\bar{x}_{latrine} = 68.3$, $\bar{x}_{random} = 68.1$), it was not biologically important in predicting latrine location because the forested sites in which the sampling was done were predominantly edge habitat. However, substrate type was an important variable for predicting latrine presence ($F = 747.46$, $P < 0.00001$).

When substrate type was omitted from the analysis, the number of overstory trees present, average stem counts and number of understory trees were important variables in classifying sites. The discriminant analysis using these variables correctly classified 93 of 132 locations (70.4%). Twenty-six of 82 latrines (32%) and 13 of 50 (26%) random locations were misclassified. Relative to random locations, latrine sites were characterized by fewer overstory trees (F = 12.85, P = 0.031, $\bar{x}_{latrine}$ = 2.23, $\bar{x}_{random}$ = 3.68), greater average stem counts (F = 4.76, P = 0.031, $\bar{x}_{latrine}$ = 3.72, $\bar{x}_{random}$ = 2.50), and more understory trees (F = 8.38, P = 0.0045, $\bar{x}_{latrine}$ = 9.24, $\bar{x}_{random}$ = 6.52).

DISCUSSION

Our results provide evidence that *Baylisascaris procyonis* eggs commonly are present at latrine sites. The low percentage of latrines with eggs present may reflect fluctuations in the prevalence of *B. procyonis* in the raccoon population, or differences in latrine usage by particular raccoons. Monitoring of latrine sites with cameras in subsequent studies documented the use of latrines by as many as three individual raccoons at one time (Page, pers. observ.).

Latrine sites differed from randomly located sites and were associated with specific structural features of wooded habitats. Latrines commonly occurred on fallen trees which created canopy gaps when they fell. Gaps are openings in a forest canopy caused by local disturbances resulting in the fall of a dominant or codominant tree (Parker and Sherwood, 1986). Canopy openings result in an increase in understory light levels (Canham, 1988) and the subsequent release of saplings to the overstory (Runkle, 1982). Our discriminant analysis of habitat features indicated a significant tendency for latrines to be located in treefall gaps.

Latrines were more often associated with substrates such as logs or stumps, increased numbers of understory trees, and higher average stem counts than random. Vertebrates concentrating activities in areas associated with such characteristics may experience elevated risks of infection if raccoons are locally abundant. Of course, food habits, mode of foraging and mode of locomotion should mediate risks associated with visiting sites that are likely to harbor raccoon latrines. We hypothesize that ground-foraging granivores, such as white-footed mice (*Peromysus leucopus*) and eastern chipmunks (*Tamias striatus*) will experience elevated risks of infection with *Baylisascaris procyonis* because they routinely incorporate fallen logs into their travel routes (Barnum *et al.*, 1992) and they apparently forage for undigested seeds present in feces at latrines (Page, pers. observ.). Similarly, granivorous birds may be drawn to latrines by the presence of undigested seeds in feces (Page, pers. observ.). Species that habitually visit logs for reasons other than foraging also may experience elevated risks of infection with *B. procyonis* due to behavior such as grooming of contaminated pelage or plumage. For example, swamp rabbits (*Sylvilagus aquaticus*) deposit their feces on the tops of stumps and logs (Terrel, 1972). If these sites also were contaminated by raccoon feces, grooming of contaminated pelage or coprophagic behavior might increase the rabbits' risk of infection with *B. procyonis*. Logs are important drumming sites for ruffed grouse (*Bonasa umbellus*) (Thompson *et al.*, 1987), and grouse may risk accidental infection by ingesting infective eggs while preening. Woodrats and other rodents which cache food and nonfood items may experience elevated risks of infection with *B. procyonis* if these items are obtained from raccoon latrines.

Humans also can contact raccoon latrine sites and become infected with *Baylisascaris procyonis*. A case of fatal infection in an Illinois child was linked to the ingestion of bark from firewood that came from downed trees in a local woodlot; presumably these logs contained raccoon latrines, because the bark was contaminated with *B. procyonis* eggs (Fox *et al.*, 1985). In another case, severe CNS disease in an infant in New York was linked to infection from contaminated soil adjacent to a large raccoon latrine under a corncrib (Cunningham *et. al.*, 1994). More casual contact of humans with these sites (*e.g.*, sitting or eating on logs or stumps) might also be important in contamination of clothes or hands with eggs, and subsequent accidental hand-to-mouth transfer. Several cases of ocular larva migrans in hunters could have occurred in such a fashion (Kazacos, pers. observ.). Absence of raccoon feces at an inactive latrine does not necessarily mean that the risk of infection has diminished, because eggs of *B. procyonis* can remain viable for years (Kazacos, 1983a; Kazacos and Boyce, 1989). Indeed, in our study there were no visible feces at a majority of latrines with eggs present. The longevity of infective eggs underscores the importance of identifying structures and habitat features associated with latrines and hence potential foci of *B. procyonis* eggs.

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