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Prevalence of *Baylisascaris procyonis* in wild rodents in central Georgia, USA

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ABSTRACT

Raccoon roundworm, *Baylisascaris procyonis,* is a zoonotic parasite of raccoons (*Procyon lotor*) that needs a One Health approach to better inform risks to human and animal health. The few studies on *B. procyonis* in wild rodents have primarily focused on white-footed mice (*Peromyscus leucopus*). This study aimed to determine the prevalence and rodent host range of *B. procyonis* in Georgia (USA) and investigate differences in prevalence at urban/fragmented sites and rural/agriculture sites. We sampled 99 rodents of five species. Larvae were recovered from seven of 78 (9.0%) white-footed mice with a mean of 4.4 larvae (range 1–12). One mouse had a single larva in the brain. Prevalence was not different between urban and rural sites. This report extends the geographic range of this parasite and confirms that rodents serve as paratenic hosts in the southern range. Therefore, baylisascariasis should be considered a differential for neurologic domestic animals, wildlife, or people in this region.

1. Introduction

Baylisascaris procyonis (raccoon roundworm) is a zoonotic parasite of raccoons (*Procyon lotor*). Because this parasite has a complex life cycle involving many wild and domestic hosts, including humans as accidental hosts, this parasite is best studied using a One Health approach. Characterizing disease risk and understanding factors contributing to the maintenance and transmission of *B. procyonis* are particularly important due to the potential for devastating larva migrans-related disease across the myriad paratenic host species. The adaptability of raccoons to human environments is a public health risk, particularly for young children who may put feces or contaminated objects into their mouths, and concerns also exist regarding its impact on ecologically important, vulnerable paratenic host species [1]. Anthropogenic factors such as raccoon or parasite introduction events and habitat changes are likely altering transmission risk across its geographic range.

The distribution of *B. procyonis* in North America is broad, with the highest prevalences reported in the Midwest, Northeast, and Western regions [1]. Historically, *B. procyonis* has been rare or absent in the southeastern US, although in recent years, infections have been reported in Georgia, North Carolina, Tennessee, and Florida [2–6], although prevalence and distribution within these states appears to be focal. Rodents are one of the more common paratenic hosts reported for *B. procyonis* and they become infected when foraging in raccoon latrine sites or by grooming contaminated fur [1,7]. Most larvae become encapsulated in organs or tissues after somatic migration, although some larvae can migrate into the eyes and cause blindness or enter the nervous tissue causing neurologic disease and potentially death [1,8]. Although clinical disease of naturally-infected rodents has been reported from dozens of species, natural asymptomatic infections have only been reported in white-footed mice (*Peromyscus leucopus)* in the Eastern United States, and deer mice (*P. maniculatus*), Western harvest mice (*Reithrodontomys megalotis)* and invasive black rats (*Rattus rattus)* in California [8–10]. Experimentally, white-footed mice can survive infections when exposure burdens are low, and they have prolonged survival times compared to other *Peromyscus* spp. (including *P. maniculatus*) and laboratory mice (*Mus musculus*) [8].

Human activity has a profound effect on habitats and these changes can affect pathogen transmission and disease risk. Studies on the impacts of urbanization on *B. procyonis* prevalence in raccoons are inconclusive

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with some studies reporting higher prevalence in raccoons and white-footed mice in urban landscapes [11,12]; while others find no relationship between landscape type and *B. procyonis* infections [13,14]. However, urban landscapes can have high densities of rodents and raccoons in small areas which could increase transmission. Habitat fragmentation can also alter infection risk [11,15].

Because few studies have investigated the prevalence of *B. procyonis* in wild rodents, and those studies in the Eastern United States focused on white-footed mice, the primary goal of this study was to determine the prevalence and host range of *B. procyonis* in rodents in the Piedmont of Georgia. In addition, we sampled urban/fragmented sites and rural/agriculture sites to investigate differences in *B. procyonis* prevalence.

2. Materials and methods

Rodents were trapped in two urban/fragmented and three rural/agriculture sites in three neighboring counties of Georgia in 2014–2015 using Sherman box traps (H.B. Sherman Inc., Tallahassee, FL, USA) baited with birdseed and dry oatmeal during summer (April–October) and birdseed and peanut butter during winter months (November–March) (Table 1; Supplemental Fig. 1). Urban/fragmented sites were defined in this study as sites that were forested areas located within 8 km of the University of Georgia (UGA)'s campus, with Whitehall Forest being an 840 acre forest with residences and University buildings in and around the forest. Our rural sites were located greater than 8 km from the UGA campus and consisted of large areas of agricultural land with some forested land on or surrounding the site. Traps were set in the evening and checked in the morning (08:00 in summer and 09:00 in winter). Rodents were anesthetized via isoflurane inhalation and euthanized by cervical dislocation followed by exsanguination (cardiac puncture). All animal capture and sampling methods were reviewed and approved by the UGA IACUC (A2014–01-007).

Immediately after euthanasia, rodents were skinned and necropsied. Each animal was examined for visible granulomas or lesions and food and fecal matter was removed from the gastrointestinal tract. To detect larvae in the brain tissue, whole brains were removed, pressed between two glass plates (12.7 mm diameter), and examined under a dissecting microscope (X30). To detect larvae in tissues, visceral organs and skeletal muscles were artificially digested together. Rodent tissues were comminuted with 100–200 mL of 1% pepsin/1% HCl in 0.85% NaCl solution using a blender (100 mL for smaller rodents, and 200 mL were used for larger rodents). The solution was stirred on a hot plate with a magnetic stir bar at 37 °C until fully digested (1–2 h for mice and 2–4 h for rats/chipmunks). When the contents were completely digested, the solution was poured through cheesecloth into a flask. The solution was allowed to settle for 15–20 min, was decanted, and then the undisturbed pellet was washed with water and allowed to settle again as described above. After decanting a second time, the remaining fluid and pellet was examined under a dissecting microscope (X30). Statistical differences were determined using Chi Squared Significance Test.

Table 1
Baylisascaris procyonis prevalence in brain and body samples from rodents from three counties (Athens-Clarke, Jackson and Oconee) in the Piedmont region of Georgia, USA.

Site	County	Classification	Species	n	No. Positive (%)	
					Brain	Body
SCWDS-RS	Athens-Clarke	Urban/fragmented	*Peromyscus leucopus*	41	1 (2)*	5 (12)
			Peromyscus polionotus	4	0	0
			Sigmodon hispidus	2	0	0
			Tamias striatus	5	0	0
			Rattus norvegicus	1	0	0
WHEF	Athens-Clarke	Urban/fragmented	*Peromyscus leucopus*	12	0	0
			Peromyscus polionotus	1	0	0
			Sigmodon hispidus	1	0	0
UGA HF	Oconee	Rural/agriculture	*Peromyscus leucopus*	1	0	0
UGARCF	Oconee	Rural/agriculture	*Peromyscus leucopus*	8	0	0
			Sigmodon hispidus	7	0	0
BC	Jackson	Rural	*Peromyscus leucopus*	16	0	2 (13)
Total				99	1*	7

* This individual was positive in both the brain and body samples.

3. Results

A total of 99 rodents of five species were sampled (78 white-footed mice, 10 cotton rats [*Sigmodon hispidus*], five oldfield mice [*Peromyscus polionotus*], five eastern chipmunks [*Tamias striatus*], and one Norway rat [*Rattus norvegicus*]) (Table 1). Larvae consistent with *B. procyonis* were recovered from seven white-footed mice (9.0% prevalence). Body tissues of infected white-footed mice had a mean of 4.4 larvae (range of 1–12). Only one white-footed mouse had a single larva detected in the brain tissue and it was the rodent with the highest burden in the body (Table 1; Fig. 1).

Infected mice were only detected at two sites (one urban and one rural), but no difference in prevalence was noted by site classification ($X^2 = 0.096$, $p = 0.7563$). Overall, at urban sites, 5/53 (9.4%) mice were positive for *B. procyonis* while 2/25 (8%) mice sampled from rural sites were positive. From the locations where infection was noted, at the specific positive sites, 14% (5/35) of white-footed mice at the urban site and 13% (2/16) at the rural site were positive. None of the rodents captured for this project showed any obvious clinical signs of infection (i.e. neurologic behavior, lethargy, scruffy coat); however, to reduce stress on animals, the time of animal handling prior to euthanasia was minimized. Therefore some animals could have been exhibiting minor signs that may have been missed.

4. Discussion

We detected *B. procyonis* in wild-caught white-footed mice in two counties in central Georgia, one of which (Jackson) represents a new county record. Four other rodent species were negative; however, sample sizes were small, and it is possible that infected individuals succumb to larva migrans or are predated quickly and thus would not be captured [1]. While artificial tissue digestion is considered a gold standard for larvae recovery from infected animals, performance for light infections can be hampered by various factors (e.g. larval viability, loss of larvae in filtration and decanting steps, etc.). Serologic testing has been proposed for overcoming the drawbacks and low sensitivity of artificial digestion or direct tissue examination methods. However, meaningful, accurate interpretation of antibody-based assays in wild rodent populations is challenging [16].

Variable susceptibility to *B. procyonis* larval migrans-related disease has been shown for several *Peromyscus* spp. with white-footed mice showing prolonged survival compared to *P. maniculatus, P. polionotus,* and *P. californicus*, regardless of dose [8]. For white-footed mice, significantly more larvae were recovered from the intestinal tract and abdominal organs showing a better ability to prevent larvae from entering the skeletal muscles and central nervous tissue. Comparatively little is known about comparative susceptibility and disease risk for other wild rodent species; however, studies have found that a variety of rodent species will forage and utilize raccoon latrine sites and rats, in

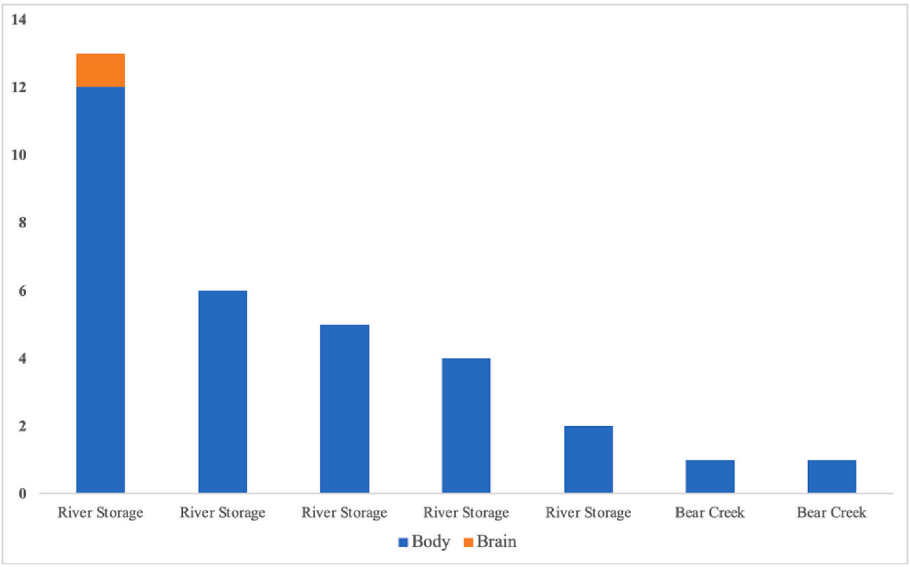

Fig. 1. Number of larvae recovered from individual *Baylisascaris procyonis*-positive white-footed mice (*Peromyscus leucopus)* sampled during the at rural (Bear Creek) and urban (River Storage) sites in Georgia, USA.

particular, using these sites commonly [17]. Additionally, there is concern for some endangered species such as Key Largo cotton mice (*Peromyscus gossypinus allapaticola*) and woodrats (*Neotoma* spp.), which are species that cache food and thus may increase risk of infection [18,19].

Habitat type can be an important factor in animal ecology and parasite transmission dynamics. Previous studies have found that urban and fragmented sites have an increased prevalence of *B. procyonis* in white-footed mice versus rural or less disturbed sites, but we did not find any difference in prevalence of larvae from rodents based on habitat [12,15]. However, we only had one positive site within each habitat type which precludes definitive comparison. Other studies on the relationship of *B. procyonis* prevalence in raccoons and habitat type have been inconclusive [13,14,20]. The impact of paratenic host ecology in this context requires further investigation.

The detection of *B. procyonis* infections in white-footed mice in central Georgia extends the geographic range of this parasite and confirms that rodents serve as paratenic hosts in its southern range. Previously, infections have only been reported in raccoons and dogs in the Southeastern United States [2–6,21]. Thus, *Baylisascaris* should be considered a differential for neurologic animals in this region. Ultimately, the contribution of paratenic hosts to the maintenance and transmission of this parasite is one such piece of the puzzle, however such studies are scarce compared to those on the racoon definitive host. To better understand the risks of this parasite to humans and animals, an interdisciplinary approach within a One Health framework is important because of the large paratenic host range, variable host-related factors related to likelihood of disease, impact of the environment on prevalence and distribution of the parasite, etc. Future studies should continue to investigate host- and environmental- impacts on the prevalence and distribution of this parasite, especially in areas where it is considered rare or emerging in the United States and internationally.

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CRediT authorship contribution statement

Kayla Garrett: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Ian Buchta:** Data curation, Investigation. **Christopher A. Cleveland:** Data curation, Investigation, Writing – review & editing. **Amanda Holley:** Data curation, Investigation. **Sarah G.H. Sapp:** Investigation, Writing – review & editing, Data curation. **Michael Yabsley:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100742.

References

[1] K.R. Kazacos, *Baylisascaris* larva migrans – circular 1412, in: United States Department of the Interior, 2016. U.S. Geological Survey.
[2] M.L. Eberhard, E.K. Nace, K.Y. Won, G.A. Punkosdy, H.S. Bishop, S.P. Johnston, *Baylisascaris procyonis* in the metropolitan Atlanta area, Emerg. Infect. Dis. 9 (12) (2003) 1636–1637, https://doi.org/10.3201/eid0912.020795.

[3] M.J. Souza, E.C. Ramsay, S. Patton, J.C. New, *Baylisascaris procyonis* in Raccoons (*Procyon lotor*) in Eastern Tennessee, J. Wildl. Dis. 45 (4) (2009) 1231–1234, https://doi.org/10.7589/0090-3558-45.4.1231.
[4] E.L. Blizzard, C.D. Davis, S. Henke, D.B. Long, C.A. Hall, M.J. Yabsley, Distribution, prevalence, and genetic characterization of *Baylisascaris procyonis* in selected areas of Georgia, J. Parasitol. 96 (2010) 1128–1133, https://doi.org/10.1645/GE-2518.1.
[5] S.M. Hernandez, B. Galbreath, F.R. Dennis, A.P. Moore, M.B. Palamar, M.G. Levy, C.S. DePerno, M.T. Correa, M.J. Yabsley, *Baylisascaris procyonis* in raccoons from North Carolina and current status of the parasite in the USA, Parasitol. Res. 112 (2) (2012) 693–698, https://doi.org/10.1007/s00436-012-3186-1.
[6] M.W. Cunningham, D.P. Wolf, K.A. Sayler, M. Milleson, B. Bankovich, P. Schueller, B.S. Haley, S. Stura, M.J. Yabsley, Distribution of *Baylisascaris procyonis* in raccoons (*Procyon lotor*) in Florida, USA, J. Wildl. Dis. 59 (2023) 347–352, https://doi.org/10.7589/JWD-D-22-00115.
[7] L.K. Page, R.K. Swihart, K.R. Kazacos, Seed preferences and foraging by granivores at raccoon latrines in the transmission dynamics of the raccoon roundworm (*Baylisascaris procyonis*), Can. J. Zool. 79 (4) (2001) 616–622, https://doi.org/10.1139/z01-024.
[8] S.G.H. Sapp, S.B. Weinstein, C.S. McMahan, M.J. Yabsley, Variable infection dynamic sin four *Peromyscus* species following experimental inoculation with *Baylisascaris procyonis*, J. Parasitol. 102 (5) (2016) 538–544, https://doi.org/10.1645/16-57.
[9] S.B. Weinstein, Introduced rats and an endemic roundworm: does *Rattus rattus* contribute to *Baylisascaris procyonis* transmission in California? J. Parasitol. 103 (6) (2017) 677–682, https://doi.org/10.1645/17-83.
[10] C.H. Sheppard, K.R. Kazacos, Susceptibility of *Peromyscus leucopus* and *Mus musculus* to infection with *Baylisascaris procyonis*, J. Parasitol. 83 (6) (1997) 1104–1111.
[11] L.K. Page, C. Anchor, E. Luy, S. Kron, G. Larson, L. Madsen, K. Kellner, T.J. Smyser, Backyard raccoon latrines and risk for *Baylisascaris procyonis* transmission to humans, Emerg. Infect. Dis. 15 (9) (2009) 1530–1531, https://doi.org/10.3201/eid1509.090128.
[12] K.F. Kellner, L.K. Page, M. Downey, S.E. McCord, Effects of urbanization on prevalence of *Baylisascaris procyonis* in intermediate host populations, J. Wildl. Dis. 48 (4) (2012) 1083–1087, https://doi.org/10.7589/2011-09-267.
[13] J.E. Jacobson, K.R. Kazacos, F.H. Montague Jr., Prevalence of eggs of *Baylisascaris procyonis* (Nematoda:Ascaroidea) in raccoon scats from an urban and a rural community, J. Wildl. Dis. 18 (4) (1982) 461–464, https://doi.org/10.7589/0090-3558-18.4.461.
[14] C.M. Jardine, D.L. Pearl, K. Puskas, D.G. Campbell, L. Shirose, A.S. Peregrine, The impact of land use, season, age, and sex on the prevalence and intensity of *Baylisascaris procyonis* infections in raccoons (*Procyon lotor*) from Ontario, Canada, J. Wildl. Dis. 50 (4) (2014) 784–791, https://doi.org/10.7589/2013-09-255.
[15] J.C. Beasley, T.S. Eagan 2nd, L.K. Page, C.A. Hennessy, O.E. Rhodes Jr., *Baylisascaris procyonis* infection in white-footed mice: predicting patterns of infection from landscape habitat attributes, J. Parasitol. 99 (2013) 743–747, https://doi.org/10.1645/GE-2887.1.
[16] S.G.H. Sapp, S. Handali, S.B. Weinstein, M.J. Yabsley, Detection and Evaluation of Antibody Response to a *Baylisascaris*-specific antigen in rodent hosts with the use of western blotting and ELISA, J. Parasitol. 104 (6) (2018) 651–659, https://doi.org/10.1645/18-48.
[17] S.B. Weinstein, C.W. Moura, J.F. Mendez, K.D. Lafferty, Fear of feces? Tradeoffs between disease risk and foraging drive animal activity around raccoon latrines, OIKOS 127 (7) (2017), https://doi.org/10.1111/oik.04866, 927–234.
[18] J. Wolfkill, M.E. Bejarano, T.L. Serfass, G. Turner, S. Brosi, D. Feller, C.G. Mahan, The prevalence of the raccoon roundworm, *Baylisascaris procyonis*, in wild Allegheny woodrat habitat in the mid-Atlantic region, U.S.A, Am. Midl. Nat. 185 (1) (2021) 145–147, https://doi.org/10.1674/0003-0031-185.1.145.
[19] K. Logiudice, Latrine foraging strategies of two small mammals: implications for the transmission of *Baylisascaris procyonis*, Am. Midl. Nat. 146 (2) (2001) 369–378. https://www.jstor.org/stable/3082917.
[20] L.K. Page, S.D. Gehrt, N.P. Robinson, Land-use effects on prevalence of raccoon roundworm (*Baylisascaris procyonis*), J. Wildl. Dis. 44 (3) (2008) 594–599, https://doi.org/10.7589/0090-3558-44.3.594.
[21] M.J. Yabsley, S.G.H. Sapp, Prevalence of *Baylisascaris* in domestic dog coprological examinations in the United States, 2013-2016, Veterin. Parasitol. Reg. Stud. Rep. 9 (2017) 65–69, https://doi.org/10.1016/j.vprsr.2017.05.003.