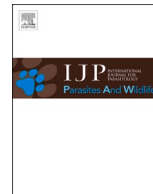




Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Demographic, environmental and physiological predictors of gastrointestinal parasites in urban raccoons

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ARTICLE INFO

Keywords:

Raccoons
Coinfection
Gastrointestinal parasites
Nematodes
Coccidia

ABSTRACT

Raccoons are host to diverse gastrointestinal parasites, but little is known about the ecology of these parasites in terms of their interactions with each other during coinfections, their interactions with host physiology and environmental factors, and their impact on raccoon health and survival. As a first step, we investigated the patterns of parasite infection and their demographic distribution in an urban-suburban population of raccoons trapped in the summers and autumns of 2018 and 2019. We collected faecal samples, demographic data, morphometric measurements, and blood smears, and used GPS data to classify trapping location by land cover type. Faecal floats were performed to detect and quantify gastrointestinal nematode eggs and coccidia oocysts, and white blood cell differentials were performed on blood smears to characterise white blood cell distributions. Data were analysed cross-sectionally and, where possible, longitudinally, using generalised linear models. Overall, 62.6% of sampled raccoons were infected with gastrointestinal nematodes, and 82.2% were infected with gastrointestinal coccidia. We analysed predictors of infection status and faecal egg count for three different morphotypes of nematode—*Baylisascaris*, strongyle, and capillariid nematodes—and found that infection status and egg count varied with Year, Month, Age class, Land cover, and coinfection status, though the significance of these predictors varied between nematode types. Gastrointestinal coccidia prevalence varied with Year, Month, Age class, strongyle infection status, and capillariid infection status. Coccidia oocyst counts were lower in adults and in October, but higher in females and in raccoons trapped in areas with natural land cover; furthermore, coccidia oocysts were positively associated with capillariid faecal egg counts. We found no evidence that gastrointestinal parasites influenced raccoon body condition or overwinter mortality, and so conclude that raccoons, though harbouring diverse and abundant gastrointestinal parasites, may be relatively tolerant of these parasites.

1. Introduction

Raccoons (*Procyon lotor*) are highly adaptable meso-carnivores found across the American continents and in introduced populations in continental Europe and Japan (Lotze and Anderson, 1979; Zeveloff, 2002). Throughout their range, raccoons live in diverse environments: from temperate forests to marshland and coastal areas (Zeveloff, 2002). They also thrive on agricultural land and in cities and reach their highest population densities in urban areas (Prange et al., 2003; Slate et al., 2020; Zeveloff, 2002).

Raccoon adaptation to urban environments poses several problems for humans. Urban raccoon populations generally have higher densities and there is potential for increased contact rates with humans (Prange et al., 2003). This has significant implications for human and domestic animal health given that raccoons serve as reservoirs of several zoonotic diseases including rabies and larval migrans disease, caused by the gastrointestinal nematode *Baylisascaris procyonis* (Sorvillo et al., 2002). These factors underscore the need to better understand disease causing organisms and zoonotic disease dynamics in these urbanized wildlife populations.

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<https://doi.org/10.1016/j.ijppaw.2023.04.011>

Received 6 September 2022; Received in revised form 28 March 2023; Accepted 27 April 2023

Available online 27 April 2023

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In addition to carrying these human diseases, raccoons are host to a diverse suite of parasites, including over twenty nematode species and several species of gastrointestinal coccidia (Inabnit et al., 1972; Weinstein et al., 2019). Though coinfections in raccoons have not been studied, multiple infections and associations between helminth species have occasionally been noted in cross-sectional studies (i.e., studies in which parasites of individual hosts are measured at a single timepoint) (Birch et al., 1994; Smith et al., 1985). Coinfecting parasites are of interest because of their ability to alter disease outcomes for individuals, and disease dynamics for populations: via their interactions, coinfecting parasites can shape susceptibility to infection, alter disease severity, and influence the distribution of parasites within populations (Ezenwa and Jolles, 2015; Telfer et al., 2010). In raccoons, such impacts of coinfections could influence the transmission of zoonotic parasites to humans. Raccoon helminths have been well surveyed in many parts of their range, but there are limited reports from New England (i.e., no reports from Vermont, New Hampshire, Maine, Massachusetts, or Rhode Island) (Weinstein et al., 2019). Raccoon gastrointestinal coccidia have been reported several times (Goodman, 2001; Monello and Gompper, 2011; Rainwater et al., 2017), but no attempts have been made to describe their epidemiology in terms of associations with raccoon demography or acquisition of immunity. This study focused on the basic principles of raccoon parasite ecology that we could compare with more well-studied host systems, such as wild rodents, lagomorphs, and ungulates (Craig et al., 2007; Fuller, 1996; Gorsich et al., 2014; Grès et al., 2003). Our research questions were: are raccoon helminths and coccidia associated with different age and sex classes, do these infections fluctuate seasonally or vary with habitat type, do these parasites have any impact on body condition or overwinter mortality, and do they interact during coinfections? To address these questions, we trapped and sampled raccoons in the summer and autumns of 2018 and 2019 in the greater Burlington area of Vermont. We analysed 610 faecal samples from 510 individual raccoons for gastrointestinal nematodes and coccidia, which represents a comparatively large sample size in raccoon parasitology. Additionally, we longitudinally tracked infection status and the intensity of shedding for nematode eggs and coccidia oocysts for 72 raccoons that were caught in July and October of the same year; this is the first such longitudinal analysis of raccoon parasites (in which parasites of individual hosts are measured over time). We use these data to investigate relationships between coinfecting parasites and between these parasites, environmental factors, and host demography.

Although our study is the first to consider all of these factors in raccoons in New England, we drew upon past work in raccoons in other locations as well as parasitological studies of other host taxa to formulate the following hypotheses. Based on previous studies in raccoons, we hypothesised that nematode infections would be more common in October than July (Kidder et al., 1989), that nematodes with direct faecal-oral transmission would be more common in urban raccoons trapped than in raccoons trapped in more natural areas (Wright and Gompper, 2005), and that nematode infections would be associated with lower body condition, and with increased overwinter mortality for juvenile raccoons (Mech et al., 1968). Based on patterns in other wildlife hosts, we hypothesised that coccidia infections would be more common and associated with higher oocyst counts in juvenile raccoons, but that they would not vary with season or host sex (Anwar et al., 2000; Grès et al., 2003). In terms of coccidia-nematode coinfections, we hypothesised that coccidia infections would be less common and associated with lower oocyst counts in raccoons coinfecting by the small intestinal hookworm *Placoconus lotoris* due to competition for intestinal epithelia (Balasingam, 1964; Dubey, 1982), but that coccidia infections would be more common and associated with higher oocyst egg counts for other coinfecting gastrointestinal nematodes due to the bystander effects of nematode immunomodulation (Su et al., 2005).

2. Methods

2.1. Study area

Trapping was conducted in the greater Burlington area of Vermont (Chittenden County). The 37 km² region trapped included parts of downtown Burlington, South Burlington, Winooski, and Colchester.

Four trapping sessions were conducted. Trapping was performed for ten consecutive days in July and ten consecutive days in October of 2018 and 2019.

2.2. Animal handling and sampling

Raccoons were trapped in tomahawk live traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) baited with marshmallows and various lures including Hard-Core Raccoon Lure (Minnesota Trapline Products, Pennock, Minnesota, USA) and anise oil.

After capture and prior to sampling, raccoons were anaesthetised with a 5:1 ratio of ketamine (10 mg/kg) to xylazine (2 mg/kg) via intramuscular injection based on their estimated body weight.

Once anaesthetised, raccoons received unique metal ear tags for future identification, and their sex, relative age (adult vs juvenile), lactation status, weight (kg), and body length (from tip of snout to base of tail) were recorded. Approximately 5 mL of blood was drawn from a jugular or peripheral vein of each raccoon, and the first pre-molar tooth was also extracted where possible (based on availability of pre-molar teeth and depth of anaesthesia). A single drop of blood was used to make a blood smear on a microscope slide. Faecal samples were collected, when possible (based on availability of faecal material in the rectum and suitable depth of anaesthesia), using disposable faecal loops (VetOne, Boise, Idaho, USA) and stored chilled in formalin.

After sampling, raccoons were returned to their traps to recover from anaesthesia, after which they were released at the point of capture. Raccoons that were recaptured within the same trapping session were released at the site without any processing after efforts were made to read and record ear tag numbers. Raccoons that were recaptured in different trapping sessions were processed as described above. Animal handling and sampling were carried out in accordance with the National Wildlife Research Center IACUC protocol QA-2942 and the Princeton University IACUC protocol 2124 F.

2.3. Parasitology

Faecal samples were processed using a modified McMaster float. Briefly, faecal samples (approximately 1–2 g per sample) were suspended in water in a 14.5 mL tube and centrifuged at 700 g for 5 min. The water was decanted and the sample was weighed before being resuspended in a saturated NaCl solution. The resuspended sample was strained through gauze, and was then used to fill both chambers of a McMaster slide. The slide was allowed to rest for 10 min before being observed under a compound microscope at 10× magnification in order to identify and count all nematode eggs and coccidia. The number of nematode eggs per gram or coccidia per gram were then calculated using the known weight of faeces, the volume of the chambers, and the volume of the NaCl solution used. We considered raccoons to be infected if we observed a single oocyst or nematode egg.

2.4. Age determination

Teeth from raccoons were sent to Matson's Laboratory LLC (Manhattan, Montana, USA) for age determination to the nearest year using the cementum annuli count method (Johnston et al., 1987).

2.5. White blood cell differentials

White blood cell (WBC) differentials were performed on a subset of

raccoons across the four trapping sessions, including samples from male, female, juvenile and adult raccoons. We used WBC differentials, or leukocyte profiles, which describe the relative proportions of different types of WBC in the bloodstream of an individual at a given point in time, as a crude picture of an individual's immune state. WBC differentials are often paired with WBC counts, though we only performed the former in this study.

Blood smears were dried, fixed with methanol, and stained with Giemsa. They were then observed under a compound microscope at 100× magnification with oil immersion, and the first 100 white blood cells were identified (as neutrophil, lymphocyte, monocyte, eosinophil, or basophil). These counts were used to calculate the ratio of neutrophils to lymphocytes (N:L ratio), and the percent of the different types of white blood cell (e.g., percent eosinophils).

2.6. Land cover analysis

We used the ESRI 2020 land use/land cover map (ESRI, 2021) to investigate the impacts of land cover type on parasite prevalence and faecal egg and oocyst counts. This dataset was downloaded to ArcGIS Pro (version 3.0) and the GPS coordinates for each raccoon's trapping location were plotted onto the land cover map, which allowed us to extract the land cover type for each location. There are ten classes of land cover in the ESRI 2020 land use/land cover map, and six of these were present in our study area (Water, Trees, Grass, Crops, Scrub, and Built Area). To simplify our analysis, we narrowed this down to three classes of land cover: "Natural" (Water, Trees, Grass, and Scrub), "Crops/Agricultural", and "Developed" (Built Area).

2.7. Data analysis

All analyses were performed in R version 3.6.0 in RStudio (RStudio Team, 2019). For all analyses, $p < 0.05$ was considered significant.

For some age class comparisons, juveniles were compared to all other age classes (juveniles vs adults; relative age), while for other analyses tooth data were used to divide the raccoons into four age classes based on a previously published system (Slate et al., 2020): age class 0 was defined as raccoons that are less than one-year old ("juveniles"); age class I was defined as raccoons that are exactly one-year old ("yearlings"); age class II was defined as raccoons that were between two and four years old ("adults"); and age class III was defined as raccoons that are five years or older ("older adults").

To determine predictors of parasite infection, we fit General Linear Models (GLMs) for each of the parasite types with parasite infection status (presence/absence) as the response variable, and Year, Month, Age, Sex, Land cover, and presence or absence of the other parasite types as possible predictors. We then used the Chi square (χ^2) univariate test to confirm associations found using the GLMs.

To determine predictors of nematode faecal egg count and coccidia oocyst count in raccoons that were infected by these parasites, we fit GLMs with negative-binomial error distributions with faecal egg or oocyst count as the response variable, and Year, Month, Age, Sex, Land cover, and presence or absence of other parasite infections as predictors. We then used Welch's *t*-test to confirm associations found using the GLMs. Note that we used the raw egg and oocyst counts for these analyses, but for easier visualisation the data were graphed using a log transformation ($\log(\text{nematode eggs per gram}) + 1$).

To explore associations between parasite infections and white blood cell differential data (e.g., the percentage of different types of white blood cell, and for associations with the neutrophil: lymphocyte ratio), we used Welch's *t*-test.

To assess body condition, we regressed \log body weight $\sim \log$ body length and then took the residuals to create a body condition index (Jakob et al., 1996). Then, we used GLMs with Gaussian error distribution to assess possible predictors of raccoon body condition, first testing all raccoons together and then testing adults and juveniles

separately. Our GLM predictors of body condition were Year, Month, Age, Sex, Land cover, and parasite infection status and egg or oocyst count. We also confirmed significant predictors in univariate models using Welch's *t*-test or a one-way ANOVA with post-hoc Tukey test.

For all GLMs, model selection was based on a backward stepwise simplification, with the gradual removal of non-significant ($p > 0.05$) variables. Models were then assessed using Akaike Information Criterion corrected (AICc) for small sample sizes to choose the best model(s); models with ΔAICc values within 2 units of the best fitting (lowest scoring) model were considered equivalent (Burnham and Anderson, 2002).

To examine how infection changed over time, we performed longitudinal analyses on raccoons that were sampled in both trapping sessions of a given year (i.e., both July and October of 2018 or 2019). For these raccoons, we compared the number of eggs and oocysts shed in July versus October using paired *t*-tests, and we calculated the percent change in infection status for each parasite (i.e., what percent remained infected, became infected, remained uninfected, or cleared infection between July and October). Finally, we calculated the change in nematode egg shedding for the different parasites by subtracting the July faecal egg count from the corresponding October faecal egg count, and compared the size of this change in egg shedding for adults vs juveniles using Welch's *t*-test.

To examine the contribution of parasite infections and coinfections (specifically, the number of types of parasite simultaneously infecting one raccoon) to overwinter juvenile mortality, we performed a similar longitudinal analysis (of change in infection status and number of eggs or oocysts shed) for nine raccoons that were trapped as juveniles in October 2018 and subsequently recaptured as yearlings in July 2019. We compared the distribution of coinfections in these raccoons that definitely survived their first winter vs all juvenile raccoons that were sampled in October 2018 to assess if coinfection, and specifically the number of different coinfecting parasites, might be influencing survival.

2.8. Sample size

We trapped a total of 1203 raccoons across all four trapping sessions and collected 610 faecal samples from 510 unique individuals. Of these 510 individuals, 417 were sampled once, 86 were sampled twice, and 7 were sampled three times. We report relative age and sex ratio for these samples in Table 1.

As we only had tooth cementum age data for 57.4% of the animals, for most analyses we grouped animals as either adults ($n = 716$) or juveniles ($n = 487$) rather than using the finer-scaled cementum age data. Of the 690 animals with cementum data, 317 were age class 0, 213 were age class I, 136 were age class II, and 24 were age class III.

For our longitudinal analyses, we analysed 144 faecal samples from the 72 raccoons that were sampled in both July and October of a given year (18 from 2018 and 54 from 2019) and 18 samples from the 9 raccoons that were sampled as juveniles in October 2018 and again as yearlings in July 2019.

3. Results

3.1. Parasitology: summary statistics

Overall, 61.3% (374/610; CI: 57.4–65.1%) of sampled raccoons were infected with gastrointestinal nematodes, 82.3% (502/610; CI: 79.1–85.1%) were infected with gastrointestinal coccidia, and 52.5% (320/610; CI: 48.5–56.4%) were coinfecting with nematodes and coccidia. Parasite prevalence and mean, minimum, and maximum egg and oocyst counts during each trapping session are shown in Table 1, and predictors of infection status and egg or oocyst count are described in section 3.2.1. for each of the different parasites. Photographs of nematode eggs and coccidia oocysts are shown in Fig. 1.

We grouped the nematodes based on their morphology, such that we

Table 1

Parasite prevalence and count data for nematode eggs and coccidia oocysts for raccoons from the greater Burlington area of Vermont for each of the four trapping sessions.

	2018		2019	
	July	October	July	October
Sample size (n)	87	127	244	152
Sex ratio (Males: Females)	50:37	63:64	123:121	85:67
Age ratio (Adults: Juveniles)	46:41	85:42	173:71	89:63
Nematode				
Prevalence (95% CI)	58.6% (48.1, 68.4)	83.5% (76.0, 88.9)	47.5% (41.4, 54.0)	66.4% (58.6, 73.5)
Mean eggs per gram (range)	49 (2–1094)	103 (1–1007)	35 (1–991)	56 (1–570)
Baylisascaris				
Prevalence (95% CI)	12.6% (7.2, 21.2)	48.0% (39.5, 56.7)	9.4% (6.4, 13.7)	37.5% (30.2, 45.4)
Mean eggs per gram (range)	107 (2–1088)	130 (4–948)	107 (1–991)	70 (3–552)
Strongyle				
Prevalence (95% CI)	43.7% (33.7, 54.1)	65.9% (57.2, 73.6)	29.1% (23.8, 35.1)	38.2% (30.8, 46.1)
Mean eggs per gram (range)	12 (2–157)	11 (1–121)	12 (2–91)	6 (1–27)
Capillariid				
Prevalence (95% CI)	10.3% (5.5, 18.5)	52.3% (43.7, 60.8)	20.9% (16.3, 26.4)	38.2% (30.8, 46.1)
Mean eggs per gram (range)	38 (2–314)	29 (1–193)	12 (1–128)	21 (2–454)
Coccidia				
Prevalence (95% CI)	77.0% (67.1, 84.6)	79.5% (71.7, 85.6)	83.7% (78.6, 87.8)	85.5% (79.1, 90.2)
Mean oocysts per gram (range)	464 (4–10507)	521 (2–19101)	755 (2–20170)	137 (2–1519)

identified four “types” of nematode egg: 1) ascarid type nematodes (likely *Baylisascaris procyonis*), 2) strongyle type nematodes (*Placoconis lotoris* or *Molineus barbatus*), 3) capillariid type nematodes (*Capillaria procyonis* or *Capillaria putorii*), and 4) strongyloides type nematodes, though only the first three were common enough to be included in our analysis. Nematode larvae, possibly from lungworms, were also noted in several of the samples.

Three different morphologies of coccidia were identified (“small”, “large”, and “long”). We grouped all coccidia together for the purpose of our analysis and refer to them simply as “coccidia”. However, given that different morphologies were observed, it is likely that this raccoon population sustains multiple species of coccidia. Two species of *Eimeria* are known from raccoons (*E. procyonis* and *E. nuttalli* (Inabnit et al., 1972; Yakimoff and Matikaschwili, 1932)), and other coccidia, including a species of *Isospora*, have been noted in the literature (Foster et al., 2004; Inabnit et al., 1972).

3.1.1. Predictors of parasite prevalence and faecal egg or oocyst count

3.1.1.1. Baylisascaris nematodes are associated with season, age, land cover, and coinfection with other nematodes. The best model of

Baylisascaris infection status included the interaction between Month and Age Class, strongyle infection status, and capillariid infection status (Tables S1 and S2). *Baylisascaris* infections were more prevalent in October than July (42.3% vs 10.3%; X-squared = 81.27, df = 1, p-value <0.0001), more common in strongyle infected raccoons (37.8% vs 15.9%; X-squared = 36.95, df = 1, p-value <0.0001), and more common in capillariid infected raccoons (44.0% vs 16.7%; X-squared = 49.94, df = 1, p-value <0.0001). Though Age class was not a significant predictor on its own in the best model, there was a significant interaction between Month and Age Class such that the increase in prevalence between July and October was more pronounced for juvenile raccoons (from 7.1% to 75.2% vs from 11.9% to 22.4% in adults). Overall, a higher proportion of juveniles were infected with *Baylisascaris* than adults (40.1% vs 116.5%; X-squared = 40.20, df = 1, p-value <0.0001).

The best model of *Baylisascaris* faecal egg count in infected raccoons included Age Class, Land cover, and strongyle infection status (Tables S3 and S4). When all other factors were controlled for, *Baylisascaris* egg counts were higher in adults than juveniles, higher in developed areas than agricultural and natural areas, and higher in strongyle infected raccoons. Though the term Month was not included in the best model (it was in a competing model), we also saw an association between Month and Age Class (Fig. 2): juvenile faecal egg counts were higher in October than July (mean egg count = 66 vs 3; Welch’s *t*-test; *t* = −5.07, df = 114, p-value <0.0001), but there was no difference in the faecal egg counts of adult raccoons between the two months (mean egg count = 15 vs 28; Welch’s *t*-test; *t* = −1.20, df = 362, p-value = 0.23).

3.1.1.2. Strongyle nematodes are associated with season, age, land cover, and coinfection with nematodes and coccidia. The best model of strongyle infection status included Year, interaction between Month and Age class, *Baylisascaris* infection status, capillariid infection status, and coccidia infection status as predictors (Tables S5 and S6). Strongyle infections were more common in 2018 than 2019 (57.0% vs 32.6%; X-squared = 33.25, df = 1, p-value <0.0001), more common in October than July (50.9% vs 32.9%; X-squared = 19.44, df = 1, p-value <0.0001), more common in *Baylisascaris* infected raccoons (62.5% vs 34.1%; X-squared = 36.95, df = 1, p-value <0.0001), and more common in capillariid infected raccoons (57.6% vs 34.0%; X-squared = 28.52, df = 1, p-value <0.0001). Though Age class was not a significant predictor on its own in the best model, there was a significant interaction between Month and Age Class such that the increase in prevalence between July and October was more pronounced for juvenile raccoons (from 19.6% to 62.9% vs from 39.7% to 43.7% in adults).

The best models of strongyle faecal egg count included Year, Sex, Month, Land cover, *Baylisascaris* infection status, capillariid infection status, and coccidia infection status as predictors (Tables S7 and S8). When other factors were controlled for, strongyle egg counts were lower in 2019, lower in October, and lower in females, but higher in agricultural areas compared to natural or developed areas, and higher in raccoons that were also infected with *Baylisascaris*, capillariid nematodes, and coccidia. Age Class was not in the best model nor was an interaction observed between Month and Age Class as observed for the other two nematode types (Fig. 2).

3.1.1.3. Capillariid nematodes are associated with season, age, land cover, and coinfection with nematodes and coccidia. The best model of capillariid infection status included the interaction between Month and Age Class, *Baylisascaris* infection status, strongyle infection status, and coccidia infection status as predictors (Tables S9 and S10). Capillariid infections were more common in October than July (44.4% vs 18.1%; X-squared = 48.54, df = 1, p-value <0.0001), more common in *Baylisascaris* infected raccoons (53.3% vs 22.5%; X-squared = 49.94, df = 1, p-value <0.0001), more common in strongyle infected raccoons (42.2% vs 21.7%; X-squared = 28.52, df = 1, p-value <0.0001), and more common in coccidia infected raccoons (33.1% vs 16.7%; X-squared =

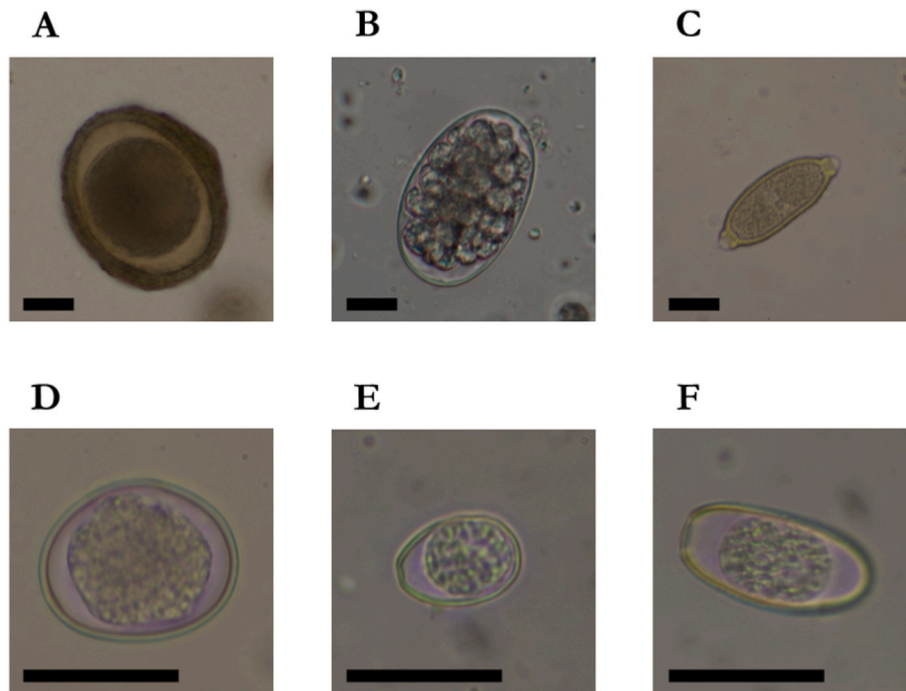


Fig. 1. Photographs of nematode eggs and oocysts taken at 40× magnification. (A) Ascarid type nematodes (likely *Baylisascaris procyonis*); (B) strongyle type nematodes (*Placoconis lotoris* or *Molineus barbatus*); (C) capillariid type nematodes (*Capillaria procyonis* or *Capillaria putorii*); (D) “large” oocysts; (E) “small” oocysts; (F) “long” oocysts. Scale bar = 20 μm in all photographs.

10.58, $df = 1$, p -value = 0.001). As seen for *Baylisascaris* nematodes, there was a significant interaction between Month and Age class such that the increase in prevalence between July and October was more pronounced for juvenile raccoons (from 0.89% to 59.0% vs from 26.9% to 35.6% in adults). Overall, there was no difference in capillariid prevalence for juveniles vs adults (29.0% vs 30.8%; X-squared = 0.130, $df = 1$, p -value = 0.719).

The best models of capillariid faecal egg count included the interaction between Age Class and Month, Year, and Land cover as predictors (Tables S11 and S12). When other factors were controlled for, capillariid egg counts were lower in 2019 and higher in natural areas. As for *Baylisascaris*, we saw an association between Month and Age Class (Fig. 2): juvenile faecal egg counts were higher in October than July (mean egg count = 21 vs 2; Welch’s t -test; $t = -3.91$, $df = 104$, p -value = 0.0002), but there was no difference in the faecal egg counts of adult raccoons between the two months (mean egg count = 4 vs 5; Welch’s t -test; $t = -0.53$, $df = 367$, p -value = 0.60). It should be noted, however, that only one juvenile raccoon was infected by capillariid nematodes in July, so the increase for juveniles may simply be a result of this very low sample size.

3.1.1.4. Coccidia are associated with season, age, land cover, and coinfection with nematodes. The best model of coccidia infection status included Year, the interaction between Month and Age Class, strongyle infection status, and capillariid infection status as predictors (Tables S13 and S14). Coccidia infections were more common in 2019 than 2018, though this was not statistically significant when tested independently (78.5% vs 84.3%; X-squared = 2.86, $df = 1$, p -value = 0.09), more common in strongyle infected raccoons, though also not statistically significant when tested independently (85.7% vs 79.9%; X-squared = 2.93, $df = 1$, p -value = 0.087), and more common in capillariid infected raccoons (90.2% vs 78.9%; X-squared = 10.58, $df = 1$, p -value = 0.001). Age was not a significant factor in isolation (prevalence in juveniles vs adults: 85.7% vs 80.4%; X-squared = 2.35, $df = 1$, p -value = 0.125). However, there was a significant interaction between Month and Age class such that coccidia prevalence was lower in October than July for

juveniles (81.9% vs 89.3%) but higher in October than July for adults (78.1% vs 83.3%), though in neither case was this difference statistically significant (X-squared; p -value > 0.1).

The best model of coccidia oocyst count included Year, Month, Age class, Sex, Land cover, and capillariid infection status as predictors (Tables S15 and S16). When other factors were controlled for, faecal oocyst count was lower in 2019 and in October, but higher in females, higher in raccoons trapped in areas with natural land cover, and higher in capillariid-infected raccoons. Again, there was an interaction between Month and Age class (Fig. 2): oocyst counts were lower in October compared to July for both adults and juveniles, but the decrease was only significant for juveniles (juveniles: mean oocyst count = 1009 vs 231; Welch’s t -test; $t = 3.25$, $df = 118$, p -value = 0.002; in adults: mean oocyst count = 319 vs 265; Welch’s t -test; $t = 0.38$, $df = 331$, p -value = 0.7).

3.1.1.5. Longitudinally sampled raccoons tend to gain nematode but not coccidia infections between July and October. Seventy-two raccoons (40 adults, 32 juveniles) provided faecal samples in July and October of the same year, allowing us to track infection status and the intensity of egg shedding between summer and autumn. Years are combined for the following analyses, though note that the lower prevalence in adult raccoons in 2019 that was uncovered during our cross-sectional analyses is still at play in these data.

Changes in infection status and mean faecal egg count for each of the three types of nematode are shown in Fig. 3. In July, 61.1% (44/72) of raccoons were uninfected by nematodes, but by October 70.5% (31/44) of these raccoons had become infected, while only 57.1% (16/28) of the raccoons that were already infected in July remained infected by October.

For coccidia infections, there was no age difference observed in terms of loss or gain of infection. In July, 18.1% (13/72) of the raccoons were uninfected with coccidia, and by October 76.9% (10/13) of these had become infected, while 86.4% (51/59) of those that were already infected in July remained infected in October (Fig. 3A).

The mean faecal egg count was higher in October than July for

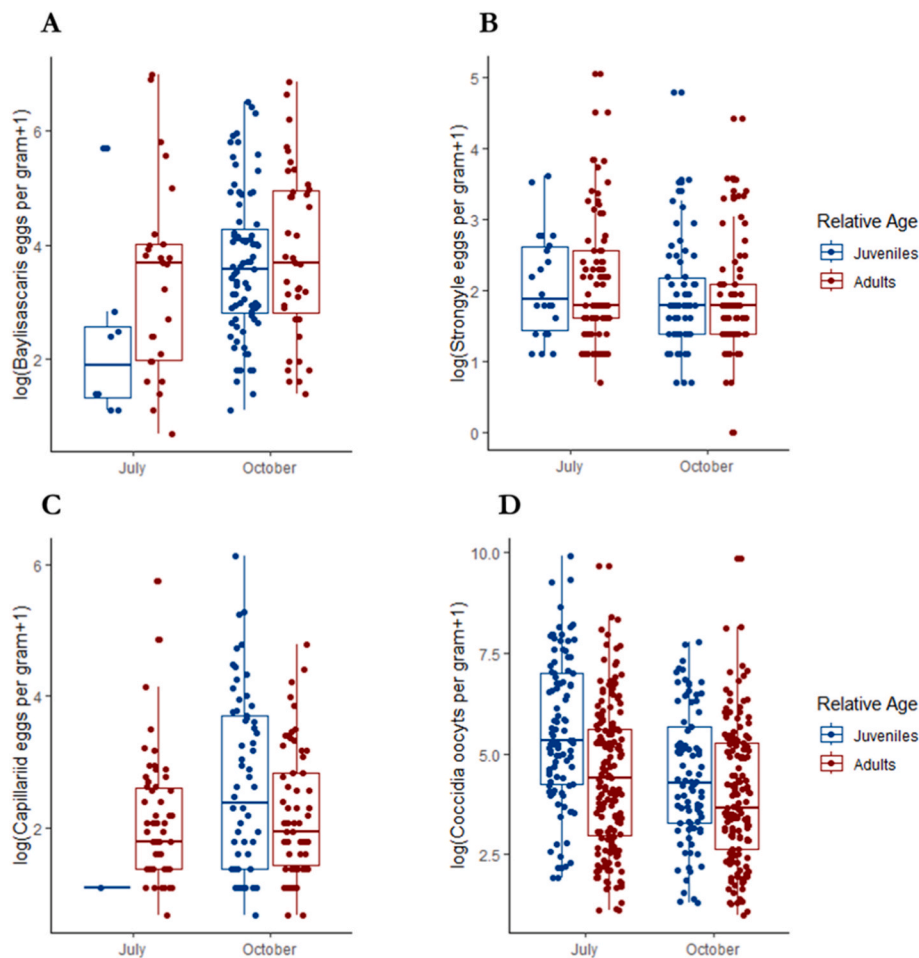


Fig. 2. Nematode and coccidia faecal egg/oocyst counts in raccoons are associated with raccoon age and the month (season) of sampling. (A) *Baylisascaris* nematodes; (B) strongyle type nematodes; (C) capillariid type nematodes; (D) coccidia.

Baylisascaris (Paired *t*-test; $t = -3.21$, $df = 71$, p -value = 0.002) and capillariid type nematodes (Paired *t*-test; $t = -3.17$, $df = 71$, p -value = 0.002), but there was no significant difference in the faecal egg count for strongyle nematode infections (Paired *t*-test; $t = -1.73$, $df = 71$, p -value = 0.087). Compared to adults, juvenile raccoons demonstrated a greater increase in faecal egg count for *Baylisascaris* (Welch's two sample *t*-test; $t = 3.09$, p -value = 0.004), strongyle (Welch's two sample *t*-test; $t = 2.02$, p -value = 0.05), and capillariid nematodes (Welch's two sample *t*-test; $t = 3.34$, p -value = 0.002).

3.1.2. Parasite coinfections are not associated with overwinter juvenile mortality

To determine whether parasite coinfections were associated with overwinter mortality, we compared the presence and distribution of coinfections (equivalent to within-host parasite richness) between juveniles in October ($n = 75$) and yearlings in July ($n = 74$). An individual raccoon could be infected by 0–4 different types of parasite: *Baylisascaris* nematodes, strongyle nematodes, capillariid nematodes, and coccidia.

From our cross-sectional data, juveniles in October had a mean of 2.8 different types of parasite, while yearlings in July had a mean of 1.4 different types of parasite (Wilcoxon rank sum test, $W = 4475$, p -value < 0.0001). Juvenile raccoons in October were more likely to harbour three or four parasites simultaneously, while most yearlings in July had two or fewer types of parasite (Fig. 4A–B).

When comparing juveniles in October with yearlings in July, significantly more juveniles were infected with nematodes (90.7% vs 55.4%; X-squared = 21.82, $df = 1$, p -value < 0.0001). When the three types of nematode were analysed separately, a significantly higher

proportion of juveniles were infected with *Baylisascaris* nematodes (77.3% vs 5.4%; X-squared = 76.38, $df = 1$, p -value < 0.0001), strongyle nematodes (61.3% vs 33.8%; X-squared = 10.26, $df = 1$, p -value < 0.0001), and capillariid nematodes (57.3% vs 28.4%; X-squared = 11.59, $df = 1$, p -value < 0.0001). However, there was no significant difference in the proportions of juveniles vs yearlings infected with coccidia (81.3% vs 79.7%; X-squared = 0.01, $df = 1$, p -value = 0.978).

The lower number of coinfections in yearling raccoons during their second summer compared with juvenile raccoons during their first fall suggests either overwinter mortality of raccoons due to parasite coinfections, or the loss or clearance of these parasites either due to acquisition of immunity or natural parasite death. To try to differentiate between these two possible explanations, we conducted a longitudinal analysis of the nine raccoons that were sampled in October 2018 as juveniles and then recaptured and sampled in July 2019 as yearlings.

We found no significant difference in the mean number of types of parasite harboured by juvenile raccoons sampled in October when we compared longitudinally and cross-sectionally sampled individuals (2.7 vs 2.8 types of parasite; Wilcoxon rank sum test, $W = 290$, p -value = 0.854; Welch's *t*-test. $t = 0.245$, $df = 8.95$, p -value = 0.812).

To investigate whether acquisition of immunity might be involved in the different distributions of coinfections for juveniles vs yearlings, we determined the change in infection status with each of the four parasites between October 2018 and July 2019 for the nine longitudinally sampled raccoons. Over this interval, raccoons tended to lose nematode infections but not coccidia infections (Table S17, Fig. 4C). Only one of the eight raccoons gained a nematode infection (a capillariid infection) between October 2018 and July 2019. Two raccoons became infected

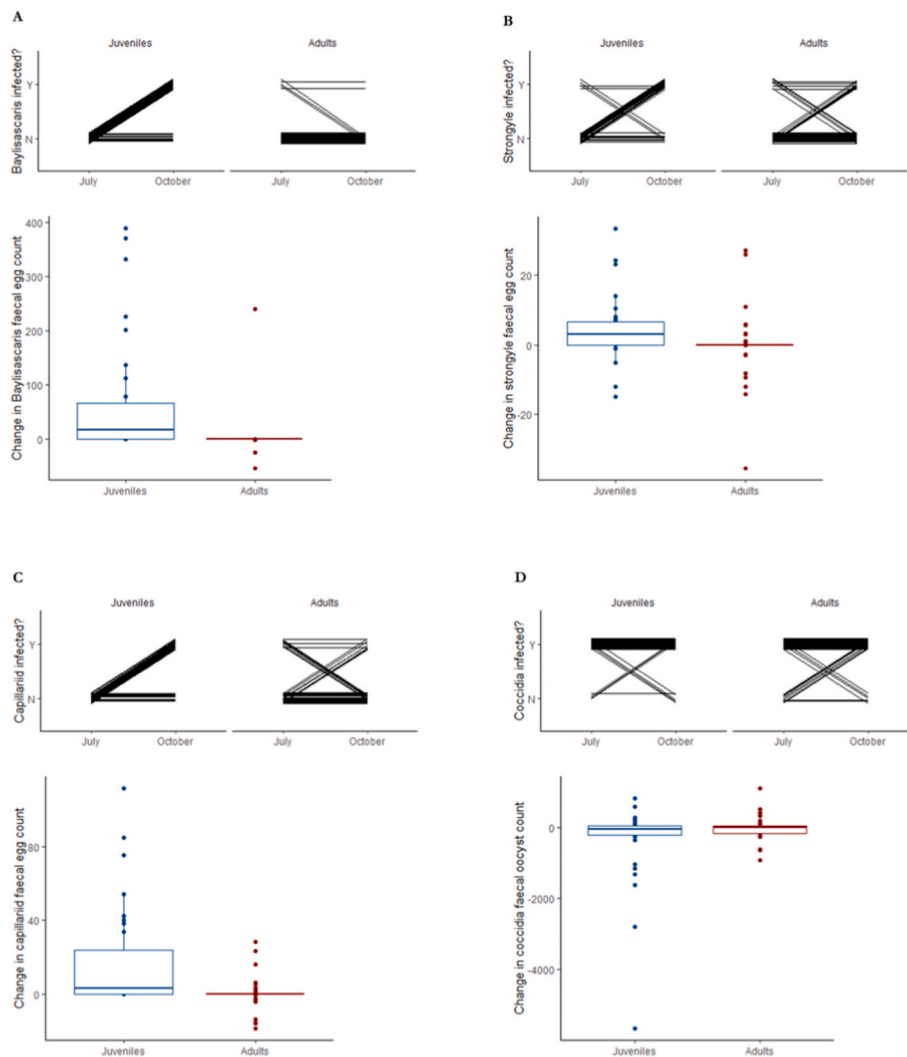


Fig. 3. Changes in gastrointestinal nematode and coccidia infection status and faecal egg or oocyst count for raccoons that were sampled in both July and October of the same year, stratified by age class. (A) Baylisascaris nematodes; (B) strongyle type nematodes; (C) capillariid type nematodes; (D) coccidia. Juvenile raccoons tended to gain nematode infections between July and October. Both adult and juvenile raccoons that were infected with coccidia in July tended to remain infected when resampled in October. Change in egg count = October egg count – July egg count. On average, the faecal egg count of juvenile raccoons increased more than the adult faecal egg count for Baylisascaris, strongyle, and capillariid nematodes (Welch’s two sample *t*-test; *p*-value <0.05), but there was no difference in the change in oocyst count for adults vs juveniles.

with coccidia during this interval, and five of the six raccoons that were infected with coccidia in October 2018 were infected in July 2019.

3.2. Raccoon body condition varies with year, month, age, sex, and land cover, but is not predicted by parasite infection status or faecal egg/oocyst count

When all raccoons were modelled together, the four best models of body condition included Year, Month, Age Class, Sex, Land cover, coccidia oocyst count, and \pm coccidia infection status and nematode infection status as predictors (Tables S18 and S19).

Age class, Sex, Month, Land cover, and Year were all statistically significant predictors of body condition in the models and when assessed independently using Welch’s *t*-test. Adults had higher mean body condition scores than juveniles (Welch’s *t*-test; $t = -2.84$, $df = 535.3$, *p*-value = 0.005), female raccoons had higher body condition scores than males (Welch’s *t*-test; $t = -3.36$, $df = 528.64$, *p*-value = 0.0008), body condition scores were higher in October (Welch’s *t*-test; $t = -8.57$, $df = 591.76$, *p*-value <0.0001), and higher in 2018 (Welch’s *t*-test; $t = 5.72$, $df = 447.78$, *p*-value <0.0001). Body condition scores were lower in areas with agricultural (Crops) land cover than in Natural areas (post-hoc Tukey test; *p*-value = 0.003), though there was no significant difference in body condition for raccoons captured in Developed areas and either Natural vs agricultural areas (post-hoc Tukey test; *p*-value >0.09) (one-way ANOVA; $F(2, 596)$, *p*-value = 0.023). Though coccidia

infection status, nematode infection status, and coccidia oocyst count were included in two of the “best” models, they were not statistically significant predictors in the models or when tested independently.

We next modelled the body condition indexes of adult and juvenile raccoons separately as the importance of other predictors may vary with age. The three best models for adult body condition included Year, Month, Sex, Land cover, and \pm coccidia infection status as predictors (Tables S20 and S21), though coccidia infection status was not a statistically significant predictor. For juvenile raccoons, the two best models included Month, Sex, Land cover, and \pm nematode infection status as predictors, though nematode infection status was not a statistically significant predictor (Tables S22 and S23).

3.3. White blood cell differentials

Our WBC differentials (from 770 blood smears) uncovered the following proportions of white blood cell: 74.1 ± 12.2 percent neutrophils; 23.7 ± 11.3 percent lymphocytes, 1.1 ± 2.6 percent monocytes; and 1.0 ± 2.3 percent eosinophils.

We found that adult raccoons had significantly higher neutrophil to lymphocyte ratios than juveniles (median = 4 vs 2.9; mean = 4.5 vs 3.6, Wilcoxon rank sum test; $W = 215$, *p*-value <0.0001). There was no significant relationship between N:L ratio and nematode or coccidia infection status, or between N:L ratio and sex.

We also performed analyses of the different types of WBC to

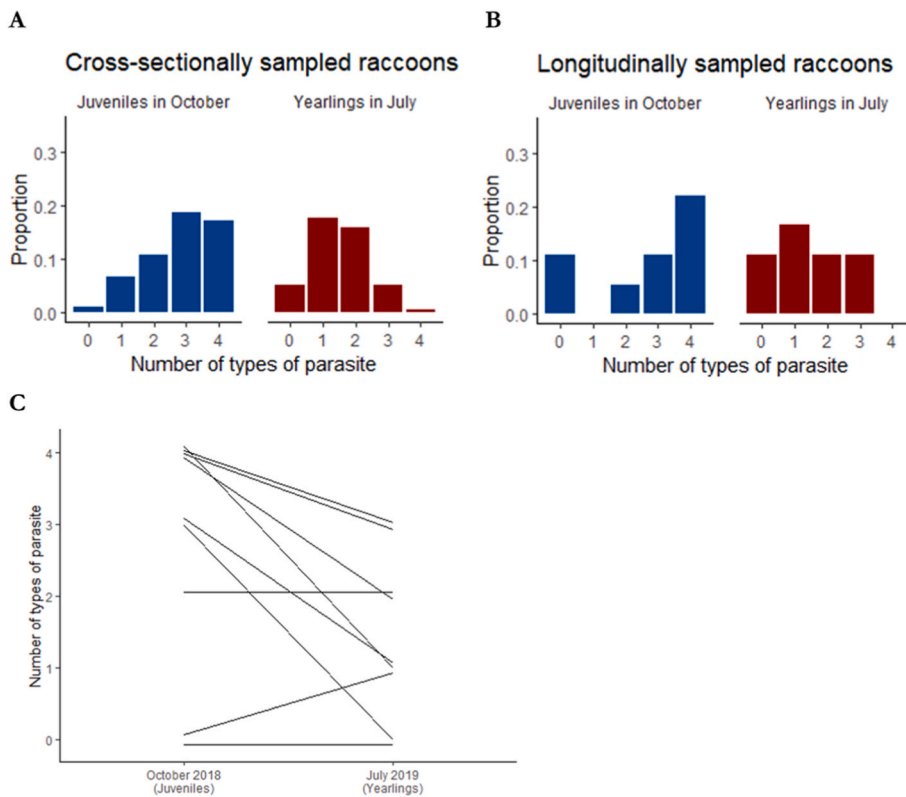


Fig. 4. Distribution of coinfections in juvenile raccoons sampled in October and yearling raccoons sampled in July. Raccoons could be infected by 0–4 types of parasite. There was no significant difference between cross-sectionally (A) and longitudinally (B) sampled raccoons in the mean number of types of parasite harboured as juveniles in October, suggesting that parasite coinfections do not contribute to overwinter mortality. However, raccoons tended to clear parasite infections rather than gain them during this interval (C).

determine whether there were any associations between the percent of these types of WBC and factors including Age, Sex, and parasite infection status and egg or oocyst count. We didn't find any associations between *Baylisascaris*, strongyle, or capillariid infection status and the percent eosinophils (Welch's *t*-test; $p > 0.05$), or the percentage of neutrophils, lymphocytes, or monocytes.

4. Discussion

4.1. Study limitations

We acknowledge a number of limitations to this study. Firstly, relying on microscopy for parasite identification resulted in less detailed groupings compared to those that could be obtained through molecular methods. Secondly, our estimates of parasite infection status and prevalence are based on faecal float data and so are subject to the usual limitations associated with this non-invasive method. Faecal floats are unable to detect non-patent nematode infections (i.e., infections where no eggs are shed), which could be due to a variety of factors; for example, if there is only one nematode present or only one sex of nematode present and hence no mating, or if the infection is too recent not yet patent due to incomplete nematode development. In terms of faecal egg count, there is the added complication that nematode egg shedding can vary with the time of day (Villanúa et al., 2006), and egg shedding is not always representative of the number of adult nematodes because of the density dependent effects of crowding on nematode fecundity (Quinnell et al., 1990). However, for *Baylisascaris procyonis* at least, other studies have found that crowding does not seem to impact fecundity, as the number of eggs shed in the faeces does correlate with the number of adult worms (Weinstein, 2016). Our estimates of coccidia prevalence and oocyst count would be similarly impacted by the within-host stage in the coccidia lifecycle; if a raccoon was only recently infected, it might not yet be shedding oocysts, or it might be shedding them at too low of a density for them to be observed in our faecal floats. Nonetheless, our methods provide a starting point for work in this

system and yielded useful comparisons with prior work, which also often relied on non-molecular methods.

4.2. Impacts of different nematode types and of nematode-nematode coinfections

Predictors of infection status and faecal egg count varied for the three nematode types. These differences may partially be explained by differences in transmission route but may also be due to within-host biology. As previously noted, *Baylisascaris procyonis* has been much more thoroughly studied than any other raccoon nematode, so it's difficult to speculate about strongyle and capillariid nematodes, and this is complicated further by the fact that what we call strongyle and capillariid nematodes are likely actually multiple species, each of which might differ in their within-host and environmental dynamics.

While we saw evidence for a strong interaction between month and age class on *Baylisascaris* and capillariid nematode infections and egg counts (i.e., for juveniles but not adults, there was a big increase in both infections and egg counts for both nematodes in October), this was not observed for strongyle nematodes. Instead, strongyle prevalence and egg counts were similar for juveniles in July and October. Our longitudinal results also supported a difference between strongyle nematodes and the other nematode types: no juvenile raccoons had patent *Baylisascaris* or capillariid infections in July, but several were already shedding strongyle eggs in July, suggesting that raccoon strongyle nematodes either have a shorter pre-patent period than the other nematode species, or they are infecting juvenile raccoons at a younger age. One of the strongyle nematodes (*Placoconus lotoris*) is thought to be capable of transmammmary transmission (Wright and Gompper, 2005), which could account for earlier infections in juveniles and therefore this lack of seasonal variation for strongyle nematodes.

We observed differences between adult and juvenile raccoons in terms of change in infection status for the different types of parasites. Adult raccoons seem to be very refractory to *Baylisascaris* infections but remain more susceptible to strongyle and capillariid nematodes.

Seasonal variation in *Baylisascaris* prevalence has been reported previously, but we are unaware of prior seasonal analyses for other raccoon nematodes, though we did hypothesise that seasonal patterns would be prevalent across these helminths. In line with our results, studies of other raccoon populations have reported higher *Baylisascaris* prevalence and burden in autumn compared to summer. A study of *Baylisascaris* in Midwestern raccoons reported that both prevalence and intensity of infection decrease from January to June and then increase from June to December (Page et al., 2016). Similarly, a report in New York state found that infection prevalence peaked in autumn (Kidder et al., 1989). Some studies have attributed this seasonal fluctuation to be a result of “self-curing” of infections over the winter combined with increased force of infection over the summer, but seasonal variation in *Baylisascaris* prevalence and burden also occurs in regions with milder climates, like California, where *Baylisascaris* prevalence peaks in autumn and winter (Weinstein, 2016). In California, this timing is attributed to the yearly timing of recruitment of highly susceptible juvenile raccoons into the population (Weinstein, 2016), an explanation that could also hold in colder climates.

The larger increase in intensity of faecal egg count for juveniles is likely because in July, most juvenile raccoons in Vermont are too young to be carrying patent (i.e., egg shedding) nematode infections, though they may be newly infected by nematodes that are yet to reach sexual maturity. For most nematode species, it takes more than a month for nematodes to reach sexual maturity and if acquired from eggs, (the main route of transmission for juvenile raccoons), *Baylisascaris* larvae take 50–75 days to reach maturity (Kazacos and Boyce, 1989). This means, in order to be shedding eggs in early July, juvenile raccoons would need to have been infected during May. Raccoon mating season varies with latitude and occurs earlier in regions of lower latitude; at latitudes comparable to Burlington, Vermont, most raccoons are born in March, April, and May, and juveniles don't begin leaving their den until ~10 weeks of age (Lotze and Anderson, 1979).

The loss of infections in longitudinally sampled raccoons suggests that raccoons might be able to mount effective immune responses and thereby clear nematode infections. This loss of nematodes was more commonly seen in longitudinally sampled adult raccoons than juveniles (Fig. 3), which fits with the paradigm that acquired immunity builds in strength with cumulative exposure to parasites (Cattadori et al., 2005). However, there are other possible explanations for loss of infections in longitudinally sampled raccoons, though they don't explain the age patterns observed. For example, nematodes may die naturally over time and not be replaced by new infections. Season might also impact the acquisition of new infections, for example by changing raccoon behaviour to make them more or less likely to encounter infectious stages, or by directly impacting the survival of infectious stages in the environment.

We observed year-to-year variation in strongyle prevalence but did not observe any yearly effect for the other nematode types. Year-to-year variation in nematode prevalence is not uncommon in wild populations and may be attributed to between-year variation in climate or host population size (Gulland and Fox, 1992). The fact that we only observed this year-to-year variation for strongyle nematodes could indicate that their eggs are more susceptible to environmental factors than *Baylisascaris* and capillariid nematodes. It should also be noted that strongyles are the only nematodes of the three that rely exclusively on direct transmission, whereas both *Baylisascaris* and capillariid nematodes can be transmitted indirectly.

We hypothesised that land cover would impact nematode prevalence and burden due to species and lifecycle-specific differences in transmission efficiency in different habitat types. Specifically, we hypothesised that nematodes with direct faecal-oral transmission (e.g., strongyle nematodes) would have increased prevalence and burden in developed urban areas, where population density and therefore contact rates are higher, while nematodes with indirect lifecycles (e.g., *Baylisascaris procyonis* and capillariid nematodes) that depend on ingestion of

an infected intermediate host would have reduced transmission and therefore reduced prevalence in urban areas where raccoons rely more on human waste for food.

Associations between land cover and raccoon nematodes are rarely reported, but two studies found higher *Baylisascaris* burdens in rural raccoons compared to urban and suburban raccoons (Page et al., 2008; Pipas et al., 2014); one of these studies also found higher prevalence in rural raccoons, while the other saw no association between land cover and prevalence. We were unable to find any analyses of associations between land cover and raccoon nematode species other than *Baylisascaris*. However, a study that experimentally increased raccoon contact rates by providing aggregated food sources (as might be found in urban settings) found that aggregation around food increased *Baylisascaris* prevalence, decreased the prevalence of the strongyle nematodes *M. barbatus* and *P. lotoris*, and had no impact on capillariid nematodes (Wright and Gompper, 2005); this study did not look at nematode egg counts, however. Though we didn't observe any associations between land cover and prevalence, we did find different associations between land cover type and faecal egg count for each of the three types of nematode: *Baylisascaris* egg counts were highest in developed areas, strongyle egg counts were highest in agricultural areas, and capillariid egg counts were highest in natural areas.

Finally, we didn't find any evidence of nematode-nematode competition, but instead found evidence that some of the nematodes might be acting synergistically; all three nematodes were more common in raccoons that were infected with other types of nematode, strongyle egg counts were higher in raccoons that also harboured *Baylisascaris* or capillariid nematodes, and *Baylisascaris* egg counts were higher in strongyle infected raccoons. These types of synergistic relationships are thought to be mediated indirectly via the immune system, since some nematodes stimulate T regulatory cells, a subset of T cells that down-regulate inflammatory responses and upregulate tolerance mechanisms (Vignali et al., 2008), allowing them to escape host immunity. The bystander effects of this modulation can benefit other parasites by allowing them to also escape immunity (Su et al., 2005).

Our finding of higher *Baylisascaris* prevalence in juveniles concurs with findings from other populations in California and New York state (Kidder et al., 1989; Weinstein, 2016). In some instances, this higher prevalence in juveniles has been associated with higher intensities of infection, but not in every case (Page et al., 2009; Snyder and Fitzgerald, 1985). Our observed prevalence of patent *Baylisascaris* infections (24.9% overall; 10.3% in July; 42.3% in October) is similar to findings from Ithaca, New York (Kidder et al., 1989), the nearest location for which prevalence has been estimated, where the overall prevalence was 20.0%, and the prevalence in the autumn was 35–48%.

4.3. Prevalence and demographic associations for coccidia infection and coccidia-nematode coinfections

While a number of studies have reported the overall prevalence of coccidia in raccoons, no previous study has compared coccidia prevalence or intensity of oocyst shedding between different age classes and sexes of raccoons, or looked at the impact of nematode coinfections. Our estimate of coccidia prevalence (82.2% overall) is at the upper end of the range of prevalence estimates previously reported for raccoon coccidia (25–82% for *E. procyonis* and 58–91% for *E. nutalli* (Adams et al., 1981; Dubey, 1982; Monello and Gompper, 2011)).

We hypothesised that coccidia infections would be more common in juvenile raccoons, and that juveniles would shed more oocysts. We found no evidence for an effect of age on coccidia prevalence, but we did observe lower oocyst counts in adults. We also observed an interaction between month and age; oocyst counts were lower in October than July, but the seasonal difference was only significant for juveniles. This could be due to the raccoons generally having better body condition in October, and thus being better equipped to combat coccidia infections.

Female raccoons shed more coccidia oocysts than male raccoons. We

were not expecting to observe a sex difference, and this difference is also contrary to what might have been predicted because, across host and parasite species, males are usually found to bear heavier parasite loads (Zuk and McKean, 1996). In the case of raccoons, this difference could be explained by the exposure of female raccoons to their kits since juvenile raccoons continue to den with their mother throughout their first autumn and winter (Gehrt and Fritzell, 1998).

Coinfections with gastrointestinal coccidia and nematodes have not been previously studied in raccoons but have been well studied in a number of herbivorous wildlife hosts (i.e., mice, rabbits, sheep and African buffalo). In these other hosts, coccidia and nematodes interact via two main types of interaction: resource mediated competition and immunomodulation. When competition is the dominant force, coccidia are less abundant in nematode-infected hosts, while nematode immunomodulation by T regulatory cells can benefit coccidia by dampening immune responses generally.

Resource mediated competition is facilitated by changes to the gastrointestinal mucosa and is very site-specific within the host, i.e., this competition is only observed when the coccidia and nematodes live within the same part of the gastrointestinal tract (Knowles et al., 2013). Raccoon *Eimeria* inhabit the small intestine (Dubey et al., 2000) alongside *Baylisascaris* and strongyle nematodes, while raccoon capillariid nematodes inhabit the stomach and oesophagus (Butterworth and Beverley-Burton, 1981). We hypothesised that we would observe a competitive interaction between coinfecting coccidia and *Placocoonus lotoris*, one of the strongyle nematodes, because coccidia rely on the small intestinal epithelia for replication, and hookworms like *P. lotoris* directly damage this epithelium (Balasingam, 1964). However, contrary to our hypothesis, we did not observe any negative impact of strongyle coinfection on coccidia oocyst counts. This could be because some or all the strongyle eggs we observed belonged to *Molineus barbatus*, a trichostrongyle that does not damage the small intestinal epithelium. Differentiation between the two possible strongyle species by molecular or other methods would be beneficial in future studies.

We found support for our alternative coinfection hypothesis — that nematode immunomodulation would positively impact coccidia infection status and oocyst counts — for capillariid nematodes but not for *Baylisascaris* or strongyle nematodes. However, this positive association could alternatively be explained by co-transmission, since coccidia are transmitted by the faecal-oral route, and capillariid nematodes can be transmitted either faecal-orally or indirectly via ingestion of intermediate hosts (Butterworth and Beverley-Burton, 1981). However given that strongyle type nematodes and *Baylisascaris procyonis* (in juvenile but not adult raccoons (Kazacos, 2001)) are also transmitted via the faecal-oral route and if the association were due to co-transmission, we would have expected to see the same relationship between coccidia and these nematodes, too.

Our finding of a positive association between capillariid nematodes and coccidia is in agreement with findings from other cross-sectional studies in Soay sheep and African buffalo that also found positive correlations between gastrointestinal nematode faecal egg counts and coccidia oocyst counts (Craig et al., 2008; Gorsich et al., 2014). However, the reliability of these cross-sectional associations has been called into question (Fenton et al., 2014), and experimental studies in wood mice (*Apodemus sylvaticus*), deer mice (*Peromyscus* sp.), and African buffalo have found the opposite type of interaction; when nematodes were experimentally removed via anthelmintic treatment, coccidia increased in prevalence and abundance (Gorsich et al., 2014; Knowles et al., 2013; Pedersen and Antonovics, 2013). Experimental anthelmintic trials in raccoons would be essential to confirming a relationship between capillariid nematodes and coccidia, and might also uncover hidden associations between coccidia and the other nematode types. Molecular (or other) definitive identification and differentiation of nematode and coccidia species would also aid future coinfection analyses.

4.4. Parasite coinfections were not associated with overwinter mortality for juvenile raccoons

It has been previously suggested that parasitism may exacerbate starvation and increase the chances of overwinter mortality for juvenile raccoons (Mech et al., 1968). However, we found no evidence to support this hypothesis. Though we did find that yearling raccoons tended to harbour fewer types of parasite compared to juvenile raccoons, our analyses suggest that this is due to loss of parasites (particularly nematodes) rather than to increased overwinter mortality in highly parasitised raccoons.

Raccoons that were captured in October as juveniles and then recaptured as yearlings the following July tended to lose nematode infections, but there was no significant change in coccidia infection status. Though helminth infections can last for years, coccidia infections are usually considered to be more acute, so these raccoons may not have been infected for that entire nine-month interval, but may instead have been reinfected in the interim. Our longitudinal analysis of raccoons that were trapped in July and October of the same year (section 3.2.1.4) also suggests that raccoons do not acquire any long-term immunity to gastrointestinal coccidia. This aligns with what is understood about immunity to coccidia in other species; a longitudinal study of coccidia in European badgers similarly found that between 49 and 88% of badgers had recovered from coccidia three months after testing positive, but that they did not acquire immunity to subsequent reinfection (Anwar et al., 2000), and recaptured flying squirrels and chipmunks showed evidence of either continuous or repeated *Eimeria* infections (Fuller and Duszynski, 1997).

The observed loss of nematode infections could be due to acquired immunity, or it could simply be a result of natural death of these parasites without reinfection, but we are unable to distinguish between these possibilities with our dataset.

4.5. Persistence of body condition despite infections and co-infections

In northern latitudes, raccoon body weight and condition tend to fluctuate seasonally, with raccoons putting on a large amount of body fat between April and October/November, and then using up those stores over the winter months (Mech et al., 1968; Stuewer, 1943). Other factors can also impact body condition, including age, sex, reproductive status, nutritional status, land use, and parasite infection status and burden (Coon et al., 2019; Moss and Croft, 1999; Sánchez et al., 2018). We found that raccoons tended to have higher body condition in October, that adult raccoons had higher body condition than juvenile raccoons, female raccoons were in better condition than males, and body condition varied with land cover. However, contrary to our hypothesis, we did not observe any significant associations between parasite infections and body condition.

Higher body condition scores in adults are not surprising, given that juvenile raccoons need to partition their energy investments between growth and energy storage, while adults only have to worry about the latter. The sex difference in body condition has been observed before; another study found that female raccoons had higher body condition than males in the autumn (Pitt et al., 2008). Furthermore, a study that experimentally manipulated the food availability for wild raccoons found that the provision of additional resources only increased the body condition of female raccoons (Monello and Gompper, 2011), suggesting that female raccoons might have some physiological advantage over males when it comes to fat storage.

Our finding of lower body condition scores in agricultural areas vs natural areas is interesting as we might expect raccoons in agricultural areas to supplement their food intake with local crops (Demeny et al., 2019). Also in contrast to our findings, a study based in southern Ontario, Canada, found that urban raccoons have better body condition than rural raccoons (Rosatte et al., 1991). This could be attributed to differences in home range size and habitat for raccoons trapped in

different areas, or to differences between the landscapes in Vermont versus Ontario. Our analysis is based only on the land cover at the trapping location, but raccoons often have large home ranges (sometimes larger than 200 ha; (Bozek et al., 2007), and these ranges could be made up of multiple land cover types and their associated food sources. It is possible that raccoons that were trapped in Natural areas that are close to urban centres might have more access to high-caloric anthropogenic food sources than raccoons whose home range is entirely agricultural.

Given that neither nematode or coccidia infection status or egg or oocyst counts were significant in any of the models, it appears that these gastrointestinal parasites are unimportant in determining body condition in raccoons; in other words, raccoons may be relatively tolerant of gastrointestinal parasite infections. Other studies of the impact of gastrointestinal nematodes on the body condition of wildlife species have found mixed results: experimental removal (anthelmintic treatment) of nematodes can improve body condition (Stien et al., 2002), or have minimal effects on body condition (Cripps et al., 2014), and this likely depends on the species of nematode involved (Budischak et al., 2018). However, cross-sectional analyses are not always able to capture these same effects (Stien et al., 2002), and so it is possible that our study design was inadequate to answer this question.

4.6. Discussion of white blood cell data

Interestingly, we didn't find any association between the percentage of different types of white blood cell and parasite infection status or egg/oocyst count. Eosinophils are associated with parasitic helminth infections (Kita, 2011), and so we expected to see higher percent eosinophils in nematode-infected raccoons, but we found no evidence of this relationship. Associations between nematodes and eosinophilia varies between nematode species and has not previously been studied in raccoons.

We observed higher neutrophil: lymphocyte ratios in adult raccoons compared to juveniles, an age-pattern that has also been observed in other wildlife species (Brandimarti et al., 2021; Carbillet et al., 2019), and in humans (Li et al., 2015). The neutrophil to lymphocyte ratio (N:L ratio, analogous to the H:L ratio in birds) is a metric that is used, both in ecoimmunology and in human medicine, as a predictor of various physiological or disease states. In wildlife ecoimmunology, high ratios of neutrophils to lymphocytes are associated with elevated stress hormones (Davis et al., 2008), and are also thought to be indicative of an inflammatory state (Davis et al., 2004), and of an active innate immune response (Dugovich et al., 2019). Adult immune systems are more developed, which could account for the age difference that we, and others, have observed.

5. Conclusions

Raccoons in the greater Burlington area of Vermont are host to at least three species of gastrointestinal nematode and two species of coccidia, though we speculate that future molecular studies may uncover cryptic species not identifiable by microscopy. Infection status and egg shedding were predicted by different factors for each of the three nematode types. We found associations of nematodes with land cover, and also evidence of synergistic nematode-nematode interactions. Coccidia infections were common, and we observed higher oocyst counts in raccoons that were coinfecting with capillariid nematodes but did not observe any associations between coccidia and the other nematode types.

Gastrointestinal parasites do not appear to be important predictors of raccoon body condition. Additionally, we found no evidence that parasite coinfections increase overwinter mortality for juvenile raccoons. Given their undetectable impact on body condition and overwinter mortality, we speculate that these gastrointestinal parasites are well-tolerated by raccoons.

Our longitudinal results suggest that raccoons may be able to clear gastrointestinal nematodes and we hypothesise that raccoons build immunity to nematodes with cumulative exposure. In contrast, we found no evidence that raccoons acquire immunity to gastrointestinal coccidia. This study provides a baseline measure of parasite ecology in raccoons that should be built upon by future studies. Future work would benefit greatly from molecular identification of raccoon parasite taxa. Longitudinal studies with additional time-points paired with experimental removal (i.e., anthelmintic treatment) of some parasite species would further improve our understanding of raccoon parasites, coinfections, and any implications these might have for wildlife or human health.

Declaration of competing interest

All authors declare that they have no conflicts of interest in the publication of this manuscript ("Demographic, environmental and physiological predictors of gastrointestinal parasite burden in urban raccoons".)

Acknowledgements

This work was supported by funds from Princeton University's Center for Health and Wellbeing and the High Meadow Environmental Institute. We are also grateful to two anonymous reviewers for their detailed and generous feedback, which greatly improved our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2023.04.011>.

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