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Baylisascaris procyonis roundworm infection patterns in raccoons (*Procyon lotor*) from Missouri and Arkansas, USA

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Summary

Baylisascaris procyonis is a helminth parasite of raccoons *Procyon lotor* and represents a health concern in paratenic hosts, including humans and diverse domestic and wildlife species. In North America the helminth is expanding its geographic range. To better understand patterns of infection in the Ozark region of the USA, raccoons (n = 61) were collected in 2013-2014 from five counties in Missouri and Arkansas, USA and necropsied. We documented *B. procyonis* in all surveyed locations. The overall prevalence of *B. procyonis* was 44.3 % (95 % CI = 31.9 – 57.4) and was significantly higher in females than males. There were also significant differences in prevalence among raccoons sampled north and south of the Missouri River. Mean intensity was 9.9 (CI = 5.44 – 17.22), and parasites were highly aggregated among hosts such that approximately 20 % of hosts harbor 90 % of parasites. These levels of parasitism indicate that *B. procyonis* is common in the region and its impacts on paratenic hosts could be qualitatively similar to effects observed in other localities.
Keywords: raccoon roundworm; Ascarididae; prevalence; intensity; Missouri; Arkansas

Introduction

The raccoon roundworm, *Baylisascaris procyonis* (order Ascaridida, superfamily Ascaridoidea), is a parasitic nematode commonly found in the small intestines of raccoons (*Procyon lotor*) (Kazacos, 2001). Larval stages of *B. procyonis* can cause clinical disease in a variety of paratenic vertebrate hosts, including humans, often resulting in fatal neurologic outcomes (Kazacos, 2001; Graeff-Teixeira *et al*., 2016). The diversity of wildlife that can become infected and the impact on some populations of hosts is increasingly recognized. For instance, Evans (2002) reported visceral, ocular, and neural larva migrans caused *by B. procyonis* in 26 species of birds and mammals from a single locale in California. Similarly, populations of the Allegheny woodrat (*Neotoma magister*), a species of conservation concern, are believed to be at risk due to severe population-scale impacts of *B. procyonis* infection (Page, 2013).

Raccoons are the definitive host for *B. procyonis*. Although raccoons are distributed across much of North America, *B. procyonis* is principally known from raccoons from the Midwest, Northeast, and West Coast states (Kazacos, 2001; Hernandez *et al*., 2013). However, the species is increasingly recorded from raccoon populations that were previously assumed to be outside the geographic range (Blizzard *et al*., 2010; Chavez *et al* 2012; Pipas *et al*., 2014). The range expansion of *B. procyonis* into new areas could have impacts on previously-unexposed paratenic host populations or species (Sapp *et al*., 2016). Such impacts on paratenic hosts populations would likely be dependent on high *B. procyonis* prevalence and infrapopulation sizes; low levels would be less likely to cause population-level effects. Problematically, documentation of the relative size of *B. procyonis* infrapopulations (that is, the inten-

sity of infection of parasitized hosts; Bush *et al*., 1997) is poorly known from regions where the parasitic nematode is newly reported. For instance, in Missouri the parasite is known to occur and in Arkansas the parasite has only recently been reported (Monello & Gompper, 2011; Al-Warid *et al*., in review), but there is little information on measures of intensity in the region. Understanding the overall pattern of *B. procyonis* prevalence and intensity in a region is a necessary first step in assessing the potential health risks for humans, domestic animals and wildlife.

Intensity information is particularly important because it allows assessment of the extent to which parasites are aggregated within the host population. Parasites often have an aggregated distribution among host individuals, which stabilizes host-parasite population dynamics (Anderson & May, 1978; Adler & Kretzscmar, 1992). Previous work on *B. procyonis* in regions the parasite has recently invaded *(e.g.* Robel *et al*., 1989; Monello & Gompper, 2011) have not fully documented the variance in intensity among individual hosts. Yet such variance can be a fundamental tenet of *Baylisascaris*-raccoon interactions. For instance, Page *et al*. (2016) included raccoon specimens collected from Missouri as part of a larger study from the Upper Midwest that reported overall prevalence of 36 % and mean intensity of 15.8, but significant overdispersion such that a relatively small proportion of hosts (7 %) had larger (>50) *B. procyonis* infrapopulations. More fully documenting aggregation patterns in raccoons could help formulate a more effective strategy for control of parasite transmission (e.g. Smyser *et al*., 2015) and predict impacts on paratenic host populations. Thus the objectives of this study were to: 1) investigate the pattern of *B. procyonis* prevalence and intensity in raccoons collected from Missouri and Arkansas, and 2) estimate aggregation indices for *B. procyonis* infection in raccoons.

Materials and Methods

We obtained 61 raccoons between November 2013 and January 2014, and in November 2014, from five counties in Missouri and one county in Arkansas (Fig. 1). Twenty-eight raccoons from Boone County, Missouri were killed by trappers for pelts, and 33 raccoons were collected by state and federal agency personnel from Cooper, Moniteau and Cole counties (Missouri) and Jefferson County (Arkansas) as a part of an unrelated study.

Raccoon carcasses were processed either fresh or after having been frozen at -20°C for up to 3 months. The sex of each raccoon was recorded and raccoons were classified as adults (≥12 mo of age) or subadults (< 12 mo) based on patterns of tooth emergence and wear. Gastrointestinal tracts were excised, opened longitudinally and nematodes, including late fourth stage *B. procyonis*, were removed and identified morphologically (Sprent, 1968; Bowman, 1987).

We calculated prevalence as the percent of examined hosts infected by *B. procyonis* (Bush *et al*., 1997). Confidence intervals (95 % CI) for prevalence were calculated using Sterne's exact method in Quantitative Parasitology 3.0 (QP 3.0; Rózsa *et al*., 2000). Differences in prevalence were examined among sexes, age classes and regions using chi-square and Fisher's exact tests. To assess regional variation, individuals were categorized as deriving from one of three regions: Central Missouri north of the Missouri River (Boone County; n = 28), Central Missouri south of the Missouri River (Cole, Moniteau, Cooper Counties; n = 27), and Northwestern Arkansas (Jefferson County; n = 6).

Intensity was defined following Bush *et al*. (1997) as the number of *B. procyonis* in an infected host, with 95 % CIs of mean intensity calculated using bootstrap tests (n = 2000; Rózsa *et al*., 2000).

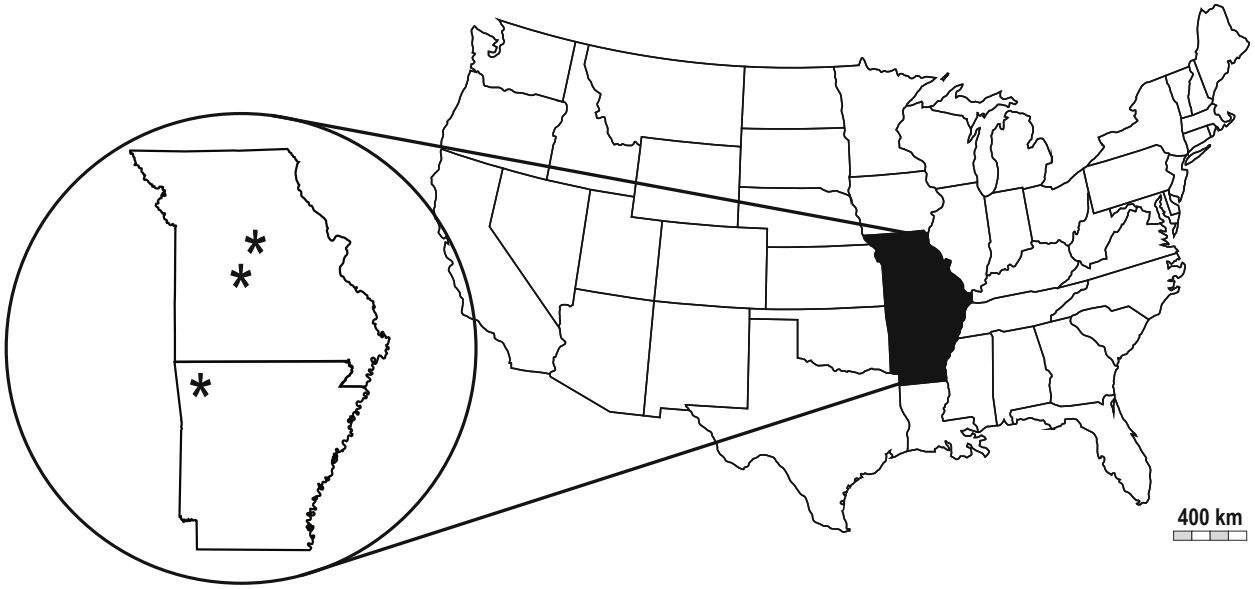

Fig. 1. Map of study sites in the central USA. Asterisks (*) indicate focal regions for specimen collection in Missouri and Arkansas

Mean intensity of regions was calculated, but statistical comparisons across regions were conducted using a Mood's median test. Aggregation of *B. procyonis* among hosts was quantified from variance/mean ratios (s^2/m) and negative binomial exponent (k) values. All statistical analyses of intensity and aggregation were conducted using QP 3.0 or the online QPweb (http://www2.univet.hu/qpweb/). To assess the relative contribution of differentially infected (or uninfected) hosts to the entire component (sensu Bush *et al*., 1997) parasite population, infrapopulations (populations of individual hosts) were ranked in decreasing order of abundance, summed, and then converted to a proportion of the component population. This value in turn was contrasted to the proportion of the contributing host population.

Results

Baylisascaris procyonis occurred in 44.3 % (CI = 31.9 – 57.4) of raccoons. Prevalence did not differ (Fisher's Exact test; p = 0.785) among adults (n = 41; prevalence = 46.3 %; CI = 31.6 – 62.3) and subadults (n = 20; prevalence = 40 %; CI = 20.9 – 62.8). However, there were differences in prevalence between males and females (Fisher's Exact test; p = 0.032). Prevalence of *B. procyonis* among male raccoons (n = 39; 33.3 %; CI = 20.3 – 50.0) was approximately half that of females (n=22; 63.6 %; CI = 41.8 – 81.3). Although samples sizes of demographic cohorts were small and overlap of CIs for prevalence was high across the cohorts, age×sex analyses indicated significant differences ($X^2 = 8.183$; df = 3; p = 0.042) in prevalence among male subadults (n = 9; 33.3 %; CI = 9.8 – 67.7), male adults (n = 30; 33.3 %; CI = 17.7 – 51.7), female subadults (n = 11; 45.5 %; CI = 20.0 – 73.5) and female adults (n = 11; 81.8 %; CI = 50.0 – 96.7), with high prevalence in adult females likely driving the differences.

Significant differences in prevalence occurred across sampling regions ($X^2 = 9.995$, df = 2, P = 0.007). Raccoons sampled from Central Missouri south of the Missouri River had higher prevalence (66.7 %; CI = 46.2 – 81.9) than those sampled from Northwestern Arkansas (33.3 %; CI = 6.3 – 72.9) and Central Missouri north of the Missouri River counties (25 %; CI = 12.0 – 44.6). While the sample size of Northwestern Arkansas animals was small, and thus the CI was large and overlapped considerably with the CI for raccoons sampled from Central Missouri south of the Missouri River, there was no overlap in the 95 % CIs for prevalence in Missouri raccoons collected north of the Missouri River and those collected south of the river.

Overall mean intensity of *B. procyonis* was 9.9 (CI = 5.4 – 17.2). For subsets of hosts (Table 1), mean intensity did not differ significantly between age classes (t = 0.079; bootstrap p = 0.939) or between the sexes (t = 1.639; bootstrap p = 0.150). There was no statistically significant difference in intensity across regions (Mood's median test; p = 0.688), although intensity south of the Missouri river had high mean intensity (12.94; CI = 6.44 – 22.78) compared with other regions, and low CI overlap with the upper limits of the 95 % CI for raccoons analyzed from north of the Missouri river (8.0) (Table 1).

For all raccoons combined, s^2/m and k values (29.65 and 0.17, respectively) indicated that *B. procyonis* was highly aggregated among hosts (Fig. 2). Relatively few hosts harbored a large proportion of the parasite population. Ranked infrapopulations indicate the six most heavily infected hosts (9.8 % of examined hosts; range in parasites per host = 16 – 54) harbored 77.5 % of the *B. procyonis* component population, and 20 % of hosts harbored 90 % of parasites (Fig. 3). Excluding uninfected hosts, 22 % of hosts were parasitized by 76 % of *B. procyonis*. Similar patterns of parasite aggregation held for host subsets (males, females, adults,

Table 1. *Baylisascaris procyonis* prevalence and mean (median for primary subsets in parentheses) intensity, with 95 % confidence interval of mean derived from 2000 bootstrap iterations, for all raccoons examined as well as subsets of the total population.

Population	n	n infected (%)	Mean intensity (median)	95%CI
all	61	27 (44.3)	9.89 (3)	5.33 – 17.04
adults	41	19 (46.3)	10.05 (3)	5.05 – 20.32
subadults	20	8 (40)	9.5 (3)	2.50 – 29.13
females	22	14 (63.6)	14.36 (3.5)	6.43 – 27.57
males	39	13 (33.3)	5.08 (2)	2.23 – 10.31
adult females	11	9 (81.8)	14.56	4.33 – 31.00
adult males	30	10 (33.3)	6	2.30 – 12.20
subadult females	11	5 (45.5)	14	2.80 – 41.00
subadult males	9	3 (33.3)	2	–
North of Missouri River	28	7 (25)	4.14 (2)	2.14 – 8.00
South of Missouri River	27	18 (66.7)	12.94 (3)	6.50 – 23.28
Arkansas	6	2 (33.3)	2.5 (2.5)	–

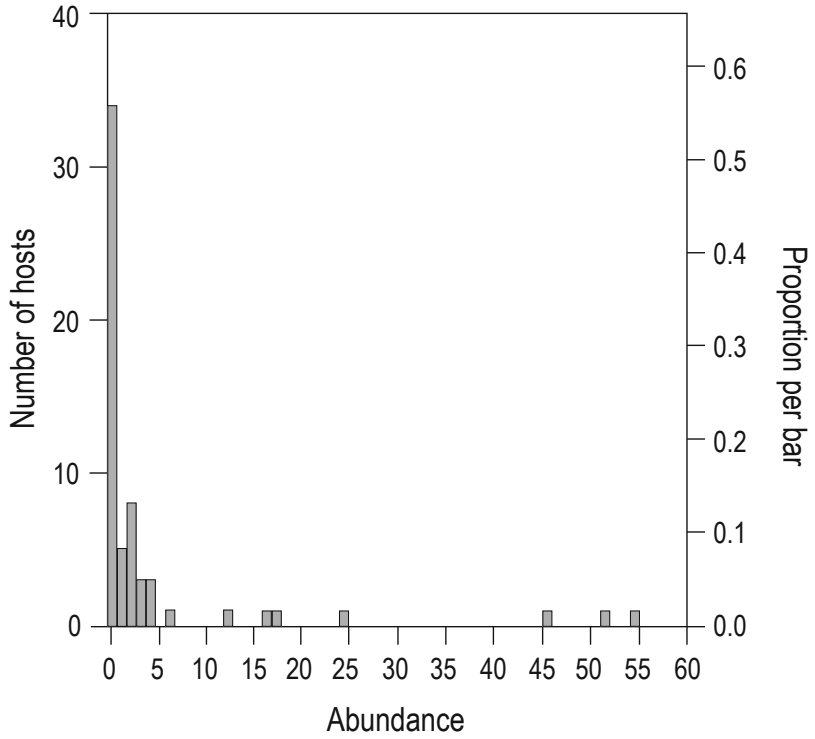

Fig. 2. Distribution of *Baylisascaris procyonis* abundance (number of parasites per host, including uninfected hosts) among n = 61 raccoons examined from Missouri and Arkansas

subadults, north of the Missouri river, south of the Missouri river); in each case there was a high level of aggregation of *B. procyonis* among hosts (Table 2). Although aggregation was greater in females than males, and south of the Missouri river compared to north of the Missouri river, in all cases the negative binomial exponents (*k*), were generally consistent with a negative binomial distribution, and in all subsets with sufficient sample sizes, the distributions were not significantly different from those predicted by the negative binomial distribution (p-values from X^2 tests were > 0.05).

Discussion

Baylisascaris procyonis has been reported from Missouri (Monello & Gompper, 2011; Page *et al*., 2016). Prevalence in central Missouri based on fecal surveys was 9 – 20 %, with the variance a function of the study population (Monello & Gompper, 2011). However, no detailed assessments of the prevalence or intensity of parasitism by *B. procyonis* based on necropsies has been previously published for the region. Further, previous work on raccoon endoparasite communities in Arkansas conducted in the 1980s and 1990s failed to identify the presence of *B. procyonis* (Richardson *et al*., 1992), which suggests the parasite may be spreading in the region. However, the detection of the parasite in Arkansas is not unexpected, as the species has been reported from most surrounding states (Hernandez *et al*., 2013). Although low numbers of raccoons were collected and only five counties surveyed from the two states,

Table 2. Aggregation metrics for *Baylisascaris procyonis* across all raccoons as well as subsets of raccoons. Higher variance/mean (s^2/m) ratios, and lower *k* values indicate an increasingly skewed distribution of parasites and increasing concentration of parasites in fewer individual hosts. Host collected from Arkansas are excluded as a distinct subset because of small sample size (n = 6).

Population	n	s^2/m	*k*
All	61	29.65	0.1721
Males	39	12.79	0.1664
Females	22	32.23	0.2665
Adults	41	28.34	0.6163
Subadults	20	34.56	0.1466
North of Missouri River	28	6.4	0.1381
South of Missouri River	27	29.96	0.2876

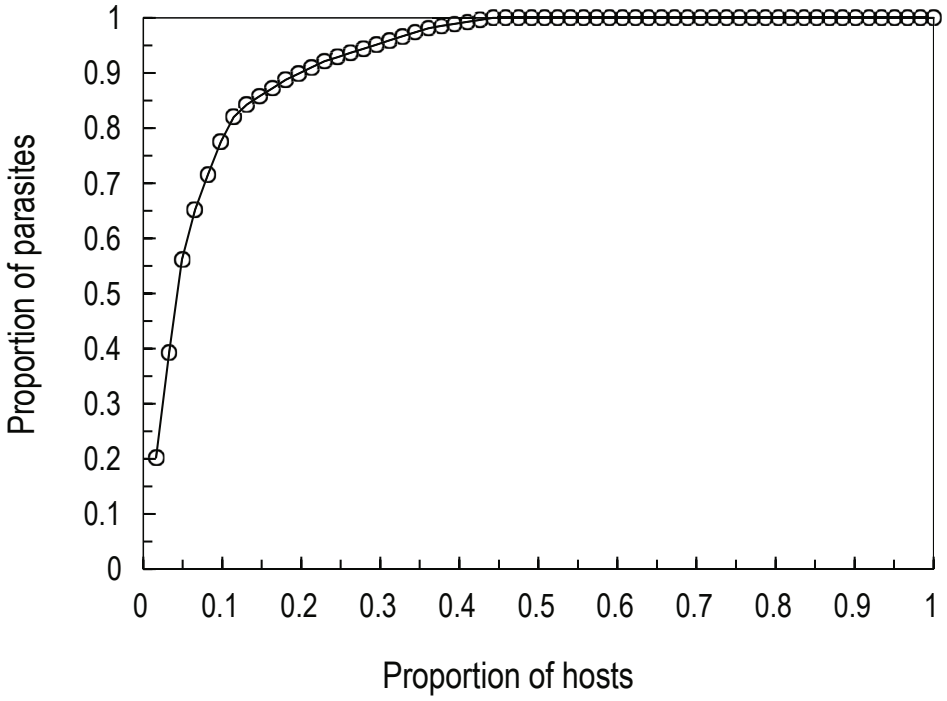

Fig. 3. Relationship between the proportion of hosts examined (n = 61) and the proportion of the total parasite component population (n = 267). Hosts are included irrespective of infection status, and are ranked in order of decreasing infrapopulation size (abundance). Approximately 20 % of the examined host population was inhabited by approximately 90 % of the parasite population

these results suggest the parasite is likely widespread in the region. The raccoons examined in this study were all collected between the months of November 2013 and January 2014, as well as during November 2014. Page *et al*. (2016) have shown strong seasonal variation in infrapopulation size of *B. procyonis* in the Midwestern US, with peaks in prevalence and intensity during approximately October – January. Similar patterns have been observed by Kidder *et al*. (1989) in New York. If such seasonal patterns also occur in the regions of Missouri and Arkansas, than our rates of observed prevalence and intensity are likely not biased downwards due to sample collection during warmer months when rates of parasitism tend to be lower. The overall prevalence observed in this study (44 %) was similar to the 36 % prevalence observed by Page *et al*. (2016). We did observe significant differences in prevalence in populations separated by the Missouri River. Such strong site-specific or fine-scale differences in the extent of parasitism are widespread in parasitology (Gibson *et al*., 2016) and have been reported for *B. procyonis* in Kansas (Robel *et al*., 1989). These patterns may be influenced by ecological factors such as habitat characteristics, and raccoon densities or contact rates (Gompper & Wright, 2005; Monello & Gompper, 2011).

The mean intensity observed in this study (9.9; CI = 5.44 – 17.22) was similar to that (15.8; 13.4 – 18.3) observed by Page *et al*. (2016). While we observed statistically significant differences in prevalence as a function of host sex and host source, such patterns were not observed for mean intensity of *B. procyonis*. This lack of robust differences in intensity when subsets of individuals were contrasted may be a function of the high rates of aggregation of parasites among hosts. While prevalence rates were relatively high, most (70 %) infrapopulations were comprised of ≤4 nematodes. In contrast, a small proportion of hosts were associated with a disproportionate number of parasites. For instance, the three most heavily infected individuals (representing 11 % of infected hosts and 5 % all hosts) accounted for 56 % of the observed parasite population. These high rates of aggregation were also observed (Table 2) when the total host population was subdivided into demographic or regional subpopulations.

Although the sample sizes of raccoons analyzed in this study are comparatively small, the resulting data allow us to emphasize several important points regarding *B. procyonis*. First, the relatively high collective rates of prevalence and intensity indicate that *B. procyonis* is common in the study region(s) and therefore the impact of this parasite on paratenic hosts including humans, domestic animals, and wildlife species may be qualitatively similar to effects observed in other locales. Second, the observed patterns of aggregation suggest the potential to use experimental and observational approaches to assess which demographic and environmental characteristics of individual raccoons best predict infection status (e.g. Monello & Gompper, 2011; Ruiz-Lopez *et al*., 2014). Such analyses are an important step in undertaking informed parasite management.

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