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Brief Report

Predicting *Baylisascaris procyonis* roundworm prevalence, presence and abundance in raccoons (*Procyon lotor*) of southwestern Ohio using landscape features

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ABSTRACT

Raccoon roundworm is a leading cause of a neurological disease known as larva migrans encephalopathy in vertebrates. We determined that roundworm prevalence is significantly lower in Beavercreek Township than other townships surveyed, and that mean patch size and proportion of landscape modified by urbanization or by agriculture are good predictors of roundworm prevalence and abundance in raccoons. The proportion of landscape modified by urbanization was the best predictor of roundworm presence. These data will facilitate predictions regarding roundworm prevalence in areas that have not been previously sampled, and contribute to devising management strategies against the spread of raccoon roundworm.

1. Introduction

The raccoon, *Procyon lotor*, has the ability to adapt to utilizing agriculture (such as corn) and other anthropogenic resources, and is distributed across North and Central America (Parsons et al., 2011). It ranges throughout rural and urban areas of North America (Parsons et al., 2011). Parsons et al. (2011) also noted that raccoons thrive in areas where there are human developments and the absence of large predators. Prange et al. (2003) reported that raccoons in urbanized landscapes had higher survival, reproductive, and recruitment rates than in rural settings. Raccoons have highest breeding success in area with large patches of forest fragmented by urbanization (Soga and Koike, 2013). These landscapes provide reliable food and denning sites. Anthropogenic resources available in urban and agricultural landscapes have allowed raccoon populations to reach higher densities compared with purely rural landscapes (Prange et al., 2003). In urban and suburban landscapes, raccoon densities can be estimated to be as high as 90 raccoons/km^2, whereas densities rarely exceeded 15 raccoons/km^2 in rural settings of North America (Prange et al., 2003). Raccoon home ranges decrease in highly fragmented landscapes, being as small as 25 hectares in some areas, yet exceeding 100 hectares in more rural areas (Beasley et al., 2007). Southwestern Ohio is a region with many areas of human developments, and few large predators. Most of the natural landscape has been converted into cultivated cropland. Within the landscape mosaic, corn is the most common crop, and provides a reliable food source for raccoons in the late summer and autumn months. The native landscape exists as small patches surrounded by agriculture and urbanization. Page et al. (2005) noted that raccoon densities are higher in urban settings, but that fewer of these raccoons are estimated to have the raccoon roundworm, *Baylisascaris procyonis*.

The Raccoon, *P. lotor*, is the definitive host for the raccoon roundworm, *B. procyonis* (see Page et al., 2005). Raccoon roundworms are the leading cause of the dangerous neurological disease known as larva migrans encephalopathy in vertebrates, found in over 90 vertebrate species (Blizzard et al., 2010b). The intermediate hosts (usually small mammals that use the raccoon feces as a source of food) are attracted to raccoon latrines, where raccoons repeatedly defecate and roundworm eggs can concentrate (Page et al., 2001b). As the density of raccoon latrines increases, the possible transmission of *B. procyonis* increases (Roussere et al., 2003). Smyser et al. (2010) noted that rather than the entire latrine,

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individual scats within a latrine should be used to assess exposure risk for intermediate hosts. Landscape fragmentation also affects the prevalence of *B. procyonis* parasites in intermediate hosts that use raccoon feces a source of food, leading to higher prevalence among raccoons preying on these hosts (Page et al., 2005). Comparisons of *B. procyonis* prevalence in raccoons and intermediate hosts tend to group the animals into areas that have drastically different landscapes and degrees of landscape fragmentation (Page et al., 2001a,b). *B. procyonis* prevalence appears to change as a function of landscape (Page, 2013). Page et al. (2001a) reported that *B. procyonis* prevalence was higher in smaller, more isolated fragments in an agricultural landscape. Page et al. (2005) found that *B. procyonis* prevalence was lower in urban portions of Chicago than in rural areas. However, Blizzard et al. (2010a) reported higher *B. procyonis* prevalence among raccoons in an urban landscape than in a rural landscape. As the prevalence of raccoon roundworm increases in raccoons, more intermediate hosts become infected, exposing more raccoons to the parasite.

The purpose of this study was to investigate if landscape features are useful predictors of both presence and abundance of *B. procyonis* among definitive hosts, and to help determine the relationship between increased anthropogenic landscape and *B. procyonis* prevalence. By testing the ability to predict the presence and abundance of parasites in a raccoons from landscape features, we aim to provide valuable information for researchers assessing the potential impact of *B. procyonis* in areas that have not yet been sampled, and for those investigators interested in the potential for zoonoses.

2. Materials and methods

We investigated raccoons from nine townships from Greene and Clark Counties in Southwest Ohio. We chose townships as the sampling scale so as to have areas larger than a typical raccoon home range in a fragmented agricultural landscape, such as that found in southwestern Ohio. The largest mean patch size in a township is less than 20 hectares (1 hectare = 10,000 m^2). With a home range of 92 ± 6 hectares for males and 58 ± 7 for females (Beasley et al., 2007), raccoons are likely to use habitat in multiple patches within a sample area. We collected raccoons from Beavercreek, Xenia, and Miami Townships in Greene County, and from German, Green, Harmony, Mad River, Moorefield, and Springfield Townships in Clark County. Municipalities were included in their respective townships for analyses (Fig. 1).

We accessed and downloaded the 2006 National Land Cover Dataset (NLCD) from mrlc.gov website. The dataset classifies land cover of each 30×30 m grid cell as belonging to one of 16 classes in eight categories: water, developed, barren, forest, shrubland, herbaceous, planted/cultivated and wetlands. Using ESRI ArcGIS software, we imported shapefile layers of Greene and Clark County townships in order to clip the NLCD. This resulted in individual land cover maps for each township. We then used Patch Analyst (http://www.cnfer.on.ca/SEP/patchanalyst/) to evaluate various landscape, class, and patch metrics.

We calculated the proportion of landscape modified by urbanization (*Turb*) and the proportion of landscape modified by agriculture (*Tag*) according to the formulas: $Turb = (Do + Dl + Dm + Dh)/TA$, and $Tag = (P + C)/TA$, where land areas are defined as: developed-open (*Do*), developed-low (*Dl*), developed-medium (*Dm*), developed-high (*Dh*), pasture/hay (*P*) and cultivated crops (*C*) for a township, and where *TA* is the total land area in the township (Table 1).

We worked with six fur trappers to gather raccoons for the study. The trappers recorded only the township, where the raccoon was trapped. We collected the viscera from trapped raccoons at two different work sites: one located in Xenia Township and one located in Harmony Township, OH (Fig. 1). We dissected out the viscera, and placed them into two freezer bags marked with the date of collection, the trapper responsible for the raccoon, the township or city where the raccoon was trapped, and the county that the township or city resides in. All samples were stored at −20 °C until they were necropsied. Collections were made from November 10 through December 9, 2012. The skinned carcasses were necropsied, and we examined sections of the gut for *B. procyonis*. We used the term abundance to refer to the number of *B. procyonis* worms present in a single raccoon whether or not it is infected (Margolis et al., 1982; Rozsa et al., 2000). We noticed that many of the necropsied raccoons also contained cestodes, so we also collected and recorded any cestodes found in the intestinal tract, but were unable to identify them to species or determine the number present in the intestines of any of the raccoons sampled. We ran a Chi-Squared equality of distributions test on the *B. procyonis* prevalence data, by combining Springfield Township with the adjacent Mad River Township (Fig 1).

We developed three models to test the correlation of *B. procyonis* features with three landscape features (*Turb*, *Tag* and mean patch size (*M*)), and the predictive capabilities of these landscape features. The first model we developed was the following linear regression: parasite prevalence $= \beta_0 + \beta_1(Turb) + \beta_2(Tag) + \beta_3(M)$, where β_0 is the intercept, and β_1, β_2 and β_3 are coefficients for the explanatory variables. We used the data from the nine townships surveyed (Table 2) for the first model. The next model tested the relationship between the three landscape features and presence of *B. procyonis*: presence $= \beta_0 + \beta_1(Turb) + \beta_2(Tag) + \beta_3(M)$, where β_0 is the intercept, and β_1, β_2 and β_3 are coefficients for the explanatory variables. This logistic regression had a binary dependent variable (either the roundworm was present or not). We ran this model on each of the 226 raccoons necropsied. The final model tested the ability of the three landscape features to predict *B. procyonis* abundance: parasite abundance $= \beta_0 + \beta_1(Turb) + \beta_2(Tag) + \beta_3(M)$. We also ran this model on each of the 226 raccoons necropsied.

After noting that many raccoons infected with *B. procyonis* were also infected with at least one cestode, we tested the ability of these landscape features to predict the cestode features: cestode prevalence in the nine townships and cestode presence in each raccoon. We used the same models as above with the cestode feature as the dependent variable. We also constructed a model to test the correlation between *B. procyonis* presence and cestode presence: roundworm presence $= \beta_0 + \beta_1$(cestode), with *B. procyonis* presence as the dependent variable and cestode representing the presence of at least one cestode at necropsy.

After preliminary analyses, we decided to drop the intercept from all of the models as the dependent variables should all be zero when all of the independent variables are zero. We added the independent variables stepwise in the linear regressions, and added the variables conditionally in the binary logistic regressions to generate the best models without introducing unnecessary variation with additional independent variables.

3. Results and discussion

We calculated the proportion of landscape modified by agriculture, and the proportion of landscape modified by urbanization for each of the nine townships (Table 1). The values for *Tag* ranged from 0.5144 in Beavercreek Township to 0.8603 in Harmony Township (Mean ± Standard Error, 0.6799 ± 0.1167). The proportion of landscape modified by agriculture exceeds 0.8 (or 80%) in two of the nine townships: Harmony and Green. We also calculated the proportion of landscape modified by urbanization

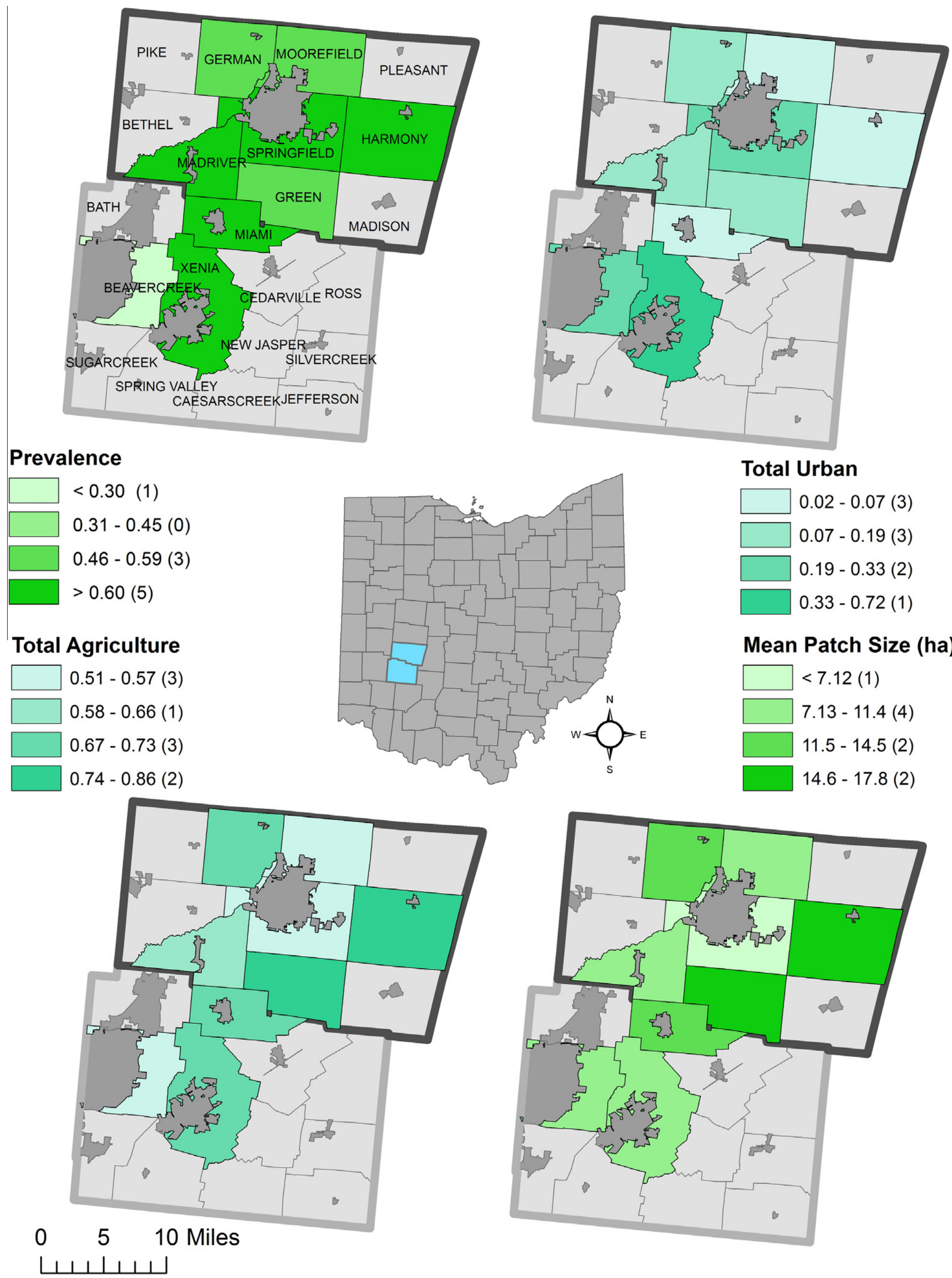

Fig. 1. Map of the townships of Greene and Clark Counties Ohio. The data represent the proportion of raccoons from an individual township that had raccoon roundworms when necropsied. This map also demonstrates the mean patch size, proportion of landscape modified by urbanization and the proportion of landscape modified by agriculture for the nine townships.

Table 1
Areas of landscape features for the nine townships surveyed in hectares (ha).[a]

Township	*Do*	*Dl*	*Dm*	*Dh*	*P*	*C*	*TA*	*Turb*	*Tag*	*M*
Beavercreek	1294	325	116	22	1332	1627	5752	0.3055	0.5144	9.2 ± 80.5
Xenia	863	365	47	9	1727	6425	11391	0.1127	0.7157	11.4 ± 64.6
Miami	403	28	7	2	736	4013	6683	0.0658	0.7106	14.5 ± 73.3
German	669	238	88	39	1415	4891	8641	0.1197	0.7298	13.0 ± 83.6
Green	815	85	37	8	830	6600	9243	0.1022	0.8039	16.8 ± 169.8
Harmony	640	213	32	5	1033	10,083	12,921	0.0689	0.8603	17.8 ± 329.0
Mad River	1055	450	66	12	1014	4562	8415	0.1881	0.6626	9.8 ± 52.6
Moorefield	729	603	168	71	1674	3270	8657	0.1815	0.5711	9.6 ± 49.5
Springfield	1912	778	140	58	936	3926	8835	0.3269	0.5503	7.1 ± 63.6

[a] (*Do*) developed-open, (*Dl*) developed-low, (*Dm*) developed-medium, (*Dh*) developed-high, (*P*) pasture/hay, (*C*) cultivated crop, (*TA*) total area, (*Turb*) proportion of landscape modified by urbanization, (*Tag*) proportion of landscape modified by agriculture and (*M*) mean patch size.

Table 2
Prevalence, mean intensity of infection and range of *B. procyonis* and prevalence of cestodes in *Procyon lotor* sampled from Greene and Clark Counties, Ohio.

County	Township	Number of raccoons	Prevalence of *B. procyonis* (%)	Mean intensity of *B. procyonis*	Range of *B. procyonis*	Prevalence of cestodes (%)
Greene	Beavercreek	49	24.5	7.00	0–52	4.2
	Xenia	37	67.6	27.40	0–176	35.1
	Miami	51	68.6	26.34	0–210	25.5
Clark	German	15	46.7	13.71	0–47	6.7
	Green	23	56.5	18.08	0–64	21.7
	Harmony	26	73.1	16.37	0–50	50.0
	Mad River	8	62.5	8.00	0–31	25.0
	Moorefield	13	46.1	3.33	0–4	0
	Springfield	4	50.0	9.50	0–15	25.0
		226[a]	54 ± 14[b]	14.41 ± 8.45[b]	0–210	22 ± 5[b]

[a] Total number of raccoons necropsied from Greene and Clark Counties, Ohio.
[b] Mean and standard error.

(Table 1). This value ranged from 0.0689 in Harmony Township to 0.3055 in Beavercreek Township (0.1635 ± 0.0965). Beavercreek Township had the highest proportion of landscape modified by agriculture and had the lowest *B. procyonis* prevalence.

We calculated the prevalence of *B. procyonis* and cestodes for all of the nine townships and both counties (Table 2). We collected 135 raccoons total from Greene County, and found *B. procyonis* individuals in 71 of those raccoons during necropsies (52.6%). The mean prevalence (55.8 ± 4.3%) is slightly higher in Clark County, although this difference was not significant ($\chi^2 = 0.737$, df = 1, $p = 0.39$). Cestode prevalence ranges from 0% in Moorefield Township to 50% in Harmony Township with a mean of 21.5% ± 0.053 S.E. As with *B. procyonis* prevalence, cestode prevalence is higher in Clark County (24.7%) than in Greene County (21.5%) although this difference was not significant ($\chi^2 = 0.320$, df = 1, $p = 0.57$). The only townships with *B. procyonis* prevalence below 50% (Beavercreek, German and Moorefield Townships) also have the three lowest cestode prevalence calculations (4.2%, 6.7% and 0% respectively).

The prevalence of *B. procyonis* from each of the various townships was determined (Table 2). The prevalence of *B. procyonis* from Beavercreek is significantly lower than the rest of the townships ($\chi^2 = 25.19$, df = 7, $p = 0.0007$). Beavercreek is the only township with *B. procyonis* prevalence below 45%. Of the 49 raccoons we necropsied, only 12 (24.5%) had raccoon roundworm.

B. procyonis prevalence increases as the proportion of landscape modified by agriculture increases ($F = 264.44$, $p < 0.001$). This finding is consistent with that reported by Page et al. (2001a) and Page et al. (2005). The agricultural landscape in southwestern Ohio is highly fragmented with small patches of agriculture and native landscape. Page et al. (2001a) found that *B. procyonis* prevalence was higher in these landscapes, and decreases from rural to urban landscapes. In contrast, Blizzard et al. (2010a) found the opposite to be true in Georgia, although the differences were not significant (12% in urban and 10% in rural). The urban areas in the Georgia study were more similar to non-urban areas and in close proximity to one another (Page, 2013).Blizzard et al. (2010a) was also studying *B. procyonis* in an area only recently documented to contain *B. procyonis* (only present in a single county of the 25 the researchers sampled from). Beavercreek Township has the lowest *B. procyonis* prevalence and the second lowest recorded *Tag*. *Tag* predicts *B. procyonis* rather well ($R^2 = 0.97$). The logistic regression designed to predict *B. procyonis* presence predicted presence better than no predictor (48% of raccoons negative and 65.5% overall vs. 0% and 54.9% for no predictor). Both mean patch size and the proportion of landscape modified by urbanization contributed to the logistic regression (Table 3). *B. procyonis* becomes more likely to be present when mean patch size increases, and less likely to be present as *Turb* increases. Beavercreek Township has the second lowest mean patch size (9.2 hectares), the second highest *Turb* (0.3055), and the lowest *B. procyonis* prevalence. These findings support our model. The abundance of *B. procyonis* in the 226 raccoons sampled correlated significantly with *Turb* and *Tag* ($F = 28.382$, $p < 0.001$). These two explanatory variables account for a small portion of the variation in abundance ($R^2 = 0.20$). The presence of cestodes at necropsy contributed significantly to the

Table 3
Regressions testing the correlation between parasite prevalence, presence and abundance and several landscape features.[a]

Dependent variable	Independent variables added stepwise or conditionally	Model independent variables[b]	β	S.E.	Test statistic	*p*-Value
Roundworm prevalence	*Turb*	*Tag*	0.814	.050	16.262	<0.001
	Tag					
	M					
Roundworm presence	*Turb*	*Turb*	−5.079	1.192	18.184	<0.001
	Tag	*M*	0.073	0.016	21.621	<0.001
	M					
Roundworm abundance	*Turb*	*Turb*	22.726	3.491	6.511	<0.001
	Tag	*Tag*	−33.892	13.776	−2.460	0.015
	M					
Cestode prevalence	*Turb*	*Tag*	0.381	0.093	4.075	0.004
	Tag					
	M					
Cestode presence	*Turb*	*Turb*	−9.690	1.323	53.605	<0.001
	Tag					
	M					
Roundworm presence	Cestode presence	Cestode	0.972	0.314	9.593	0.002

[a] All of the independent variables were added stepwise to determine the final model. *Tag* = Proportion of landscape modified by agriculture; *Turb* = Proportion of landscape modified by urbanization; *M* = Mean patch size. The final model was determined to be the model that explained the most variation in the dependent variable without adding additional variation. The two models for prevalence were linear regressions, and the remaining models were logistic regressions.
[b] These were the independent variables that contributed to the best model.

logistic regression predicting *B. procyonis* prevalence (score = 10.373, $p = 0.001$), and predicted *B. procyonis* presence better than no predictor (86.3% of raccoons negative and 55.3% overall vs. 0% and 54.9% for no predictor). Many of the raccoons positive for *B. procyonis* at necropsy were also positive for cestodes.

Cestode prevalence also increases with *Tag* ($F = 16.605$, $p = 0.004$). Much of the variation in cestode prevalence is predicted by variation in *Tag* ($R^2 = 0.63$). While all three landscape features contributed significantly to the model, only *Turb* remained after the independent variables were all added conditionally for predicting cestode presence. *Turb* predicted cestode presence better than no predictor (100% of raccoons negative and 77.4% vs. 0% and 22.6% for the model with no predictor).

We found raccoon roundworm prevalence ranged from 24.5% to 73.1% in the area of the current study. Prevalence of raccoon roundworm in this area is lower than what has been reported for similar areas, suggesting the need for further research to determine reasons for the lower prevalence in the Ohio region. Page et al. (2005) reported a documented prevalence range of 68–82% in the Midwestern United States. It is possible that prevalence can be impacted by life history stage. Raccoon resistance to *B. procyonis* is believed to increase with age, possibly lowering prevalence and mean intensity of infection (Owen et al., 2004). We did not estimate the age of raccoons we sampled, thus age cannot be ruled out as a contributing factor to the lower prevalence numbers. However, trappers informed us that most raccoons caught this year were adults. Additional factors should be explored that may predict the presence of *B. procyonis* in raccoons, as well as the overall load raccoons carry, and that may reduce the potential impact caused by the roundworm in intermediate hosts.

While the most reliable method to calculate prevalence of *B. procyonis* in *P. lotor* is to necropsy individual raccoons, this method is labor intensive, time consuming, and potentially dangerous due to zoonosis caused by *B. procyonis* (Blizzard et al., 2010b). The most convenient (although still dangerous) method to estimate prevalence of the parasite is to sample latrines used by raccoons, yet this method can underestimate prevalence (Page et al., 2005). Each female worm can produce hundreds of thousands of eggs each day, which can remain viable at latrine sites for several years (Page et al., 2011). The current study demonstrates that we can predict the prevalence, presence, and abundance of roundworms found in a raccoon based on the amount of landscape that is modified as agriculture or urban, and the mean patch size. This study also demonstrates evidence that *B. procyonis* prevalence increases as landscape use changes from urban to non-urban landscapes.

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