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EVALUATION OF ANTHELMINTIC FISHMEAL POLYMER BAITS FOR THE CONTROL OF *BAYLISASCARIS PROCYONIS* IN FREE-RANGING RACCOONS (*PROCYON LOTOR*)

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ABSTRACT: *Baylisascaris procyonis* is a common gastrointestinal parasite of raccoons (*Procyon lotor*) and is a zoonotic helminth with the potential to cause severe or fatal infection. Raccoons thrive in human-dominated landscapes, and the fecal-oral transmission pathway and lack of effective treatment make *B. procyonis* a serious threat to public health. The distribution of medicinal baits has emerged as a socially acceptable and cost-effective method for managing disease in free-ranging wildlife. We assessed the suitability of a mass-producible anthelmintic bait for *B. procyonis* mitigation by evaluating the willingness of free-ranging raccoons to consume anthelmintic baits and determining whether bait consumption successfully cleared *B. procyonis* infections from raccoons. Anthelmintic baits were modified from standard fishmeal polymer baits, the food attractant commonly used in oral rabies vaccine baits, with the introduction of 220 mg of pyrantel pamoate into the fishmeal mixture. We captured 16 naturally infected raccoons, presented one anthelmintic bait, and monitored *B. procyonis* infection over 90 d by screening feces for eggs. Treatment cleared *B. procyonis* infections for nine of 12 raccoons that consumed >10 g of the 15 g bait. We used remote cameras to monitor in situ patterns of bait consumption for anthelmintic baits relative to standard baits. Both anthelmintic and standard baits were rapidly consumed, with no differences in the rate of consumption between bait types. However, after bait contact, raccoons demonstrated a greater willingness to consume standard baits while ignoring anthelmintic baits more frequently ($P=0.06$). Initial trials of anthelmintic baits show promise, although refinement in both dose and palatability is needed. At mass production scales, the addition of pyrantel pamoate to fishmeal polymer baits would be inexpensive, potentially making anthelmintic baits a viable management option when coupled with an oral rabies vaccine or used independently for *B. procyonis* mitigation.

Key words: Anthelmintic, baiting, *Baylisascaris procyonis*, *Procyon lotor*, raccoon, zoonosis.

INTRODUCTION

Baylisascaris procyonis is a helminthic zoonosis that can cause severe or fatal infection (Sorvillo et al. 2002; Gavin et al. 2005). As a common gastrointestinal parasite of raccoons (*Procyon lotor*), the threat posed by *B. procyonis* to human health extends throughout the distribution of this widespread generalist omnivore, including the raccoon's natural (Central and North America) and introduced (Europe) range (Gavin et al. 2005; Bauer 2013; Page 2013). Mature female *B. procyonis* are prolific egg producers, with eggs disseminated into the environment through raccoon feces (Page et al. 1998, 1999; Kazacos 2001). Raccoons preferentially defecate at specific microsites (latrines) typically associated with the base of large trees, horizontal substrates (e.g., roofs or fallen logs), or near denning areas (Page et al. 1998; Bauer 2013). Millions of eggs may accumulate at latrines and on surrounding substrates, which serve as foci for infection of human and paratenic hosts. We use paratenic to describe the role of a broad array of small mammals and birds in the lifecycle of *B. procyonis*, given that

B. procyonis likely undergoes development to third-stage larvae while in the egg, like other Ascaridinae (Sprent 1992) and can directly infect the definitive raccoon host through the ingestion of these infective eggs (D. Bowman pers. comm.; Page et al. 1999; Kazacos 2001). Eggs are resilient in the environment and, in appropriate substrates, remain viable for many years (Kazacos 2001). Therefore, latrines may serve as a source of environmental contamination even in the absence of apparent raccoon feces. Following the incidental ingestion of larvated eggs, the eggs hatch and larvae begin aggressive somatic migration with human disease attributable to the invasion of the viscera, eye, or central nervous system (Sorvillo et al. 2002).

Raccoons are synanthropic, readily adapting to human-dominated landscapes. Densities in agricultural, suburban, and urban landscapes often exceed those in natural habitats (Page et al. 2001; Hadidian et al. 2010). The ability of raccoons to thrive in human-dominated landscapes, the fecal-oral transmission pathway, and lack of effective treatment options make *B. procyonis* a serious public health threat. Since *B. procyonis* was described as a causative agent of human disease (Huff et al. 1984), at least 30 severe or fatal infections have been reported (Gavin et al. 2005; Haider et al. 2012; Hernandez et al. 2013). Furthermore, the severity of this threat appears to be increasing with the geographic expansion of *B. procyonis* and recent increases in severe infection (Blizzard et al. 2010; Chavez et al. 2012; Hernandez et al. 2013; Pipas et al. 2014).

The distribution of vaccine-laden or medicinal baits is often used to manage some zoonotic diseases among free-ranging wildlife, and such disease-management efforts may be appropriate for *B. procyonis* mitigation. Oral rabies vaccination (ORV) has been used to eliminate rabies from numerous wildlife, including red fox (*Vulpes vulpes*) populations in Western and Central Europe and Ontario, Canada (MacInnes et al. 2001; Freuling et al. 2013) and coyote (*Canis latrans*) populations in south Texas (Sidwa et al. 2005). Most notably, widespread annual distribution of ORV baits is the primary tool in an active management program that seeks to prevent the expansion of the raccoon rabies variant enzootic region beyond the eastern US (Slate et al. 2009). Additionally, medicinal anthelmintic baits have been used in numerous landscape-scale field trials in Europe and Japan to control *Echinococcus multilocularis*, a common zoonotic helminth of red foxes and the causative agent of human alveolar echinococcosis (Tsukada et al. 2002; Hegglin et al. 2003; Hegglin and Deplazes 2013). Using baits to treat free-ranging wildlife for localized control of a parasitic agent poses unique challenges relative to protecting susceptible individuals with vaccine-laden baits. Unlike vaccines, consumption of an anthelmintic bait conveys no protective immunity because hosts remain susceptible to reinfection; furthermore, in the complex life cycle of a parasite such as *E. multilocularis*, environments contaminated with infective eggs or metacestode stages within intermediate host populations serve as disease reservoirs that are unaffected by anthelmintic baiting (Hegglin and Deplazes 2013). Therefore, repeated baiting is needed to obtain management objectives.

Building on established methods of using anthelmintic baits to manage parasitic zoonoses and the development of bait delivery systems attractive to raccoons, preliminary experiments have extended the application of anthelmintic baits to manage *B. procyonis* infection in free-ranging raccoons (LoGiudice 1995; Page et al. 2011; Smyser et al. 2013). Field trials employed a fishmeal polymer attractant (Bait-Tek, Orange, Texas, USA) that was developed for delivery of an oral rabies vaccine (RABORAL V-RG® fishmeal block; Merial, Duluth, Georgia, USA) and was readily accepted by raccoons relative to other bait types (Hanlon et al. 1989). Baits were modified from ORV

baits in that the hollow chamber of the attractant was filled with a dose (90 mg [base]) of the general anthelmintic pyrantel pamoate mixed with marshmallow cream and sealed within the chamber with melted paraffin wax (Page et al. 2011; Smyser et al. 2013). The monthly distribution of anthelmintic baits successfully reduced levels of environmental contamination as measured by the prevalence of *B. procyonis* eggs in raccoon feces found at latrines (Page et al. 2011; Smyser et al. 2013). Furthermore, Page et al. (2011) observed reduced *B. procyonis* infection of paratenic hosts (white-footed mice [*Peromyscus leucopus*]) within treated areas, suggesting that repeated bait distribution disrupted pathogen transmission dynamics. The multiple components of these baits (fishmeal attractant, anthelmintic, marshmallow cream, paraffin wax) make them expensive and labor intensive to assemble, limiting their suitability for broad-scale management applications. By integrating an anthelmintic directly into the fishmeal attractant, a bait could be produced that is cost effective and suitable for mass production. However, questions remain as to whether the bait manufacturing process would compromise the efficacy of the drug (Johnston et al. 2005) and if the introduction of a drug into the fishmeal attractant would reduce the willingness of raccoons to consume the bait (Hanlon et al. 1989). We evaluated the suitability of an anthelmintic fishmeal polymer bait (anthelmintic bait) for *B. procyonis* mitigation by examining 1) whether the consumption of an anthelmintic bait would successfully clear *B. procyonis* infection in naturally infected raccoons and 2) the willingness of free-ranging raccoons to consume anthelmintic baits relative to standard fishmeal polymer baits.

MATERIALS AND METHODS

Anthelmintic fishmeal polymer baits

Anthelmintic baits were modified from standard fishmeal polymer baits (Bait-Tek) with the introduction of pyrantel pamoate directly into the fishmeal matrix. Pyrantel pamoate was effective for clearing *B. procyonis* infections in naturally infected raccoons when delivered at 20 mg/kg (Kazacos 1986; Bauer and Gey 1995). Pyrantel pamoate has a wide margin of therapeutic safety, making it an appropriate drug for introduction into the environment. Anthelmintic baits were produced for this experiment by Bait-Tek using standard production protocols with a modified bait composition in which pyrantel pamoate (Sigma-Aldrich Corporation, St. Louis, Missouri, USA; 4.3% by weight) was added to the standard bait mixture (fishmeal, binder agent, and fish oil). We designed baits to deliver a therapeutic dose for raccoons in the 95th percentile by weight (11 kg; S. D. Gehrt pers. comm.). Accordingly, each 15-g anthelmintic bait delivered 220 mg of pyrantel (base).

Captive trials

We used live traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) baited with commercial cat food to collect 21 adult raccoons from Larimer County, Colorado, over a 4-wk period starting in June 2013; individuals <5 kg or actively lactating were released at the capture location. We anesthetized captured raccoons with ketamine/xylazine (10 mg/kg ketamine and 2 mg/kg xylazine, intramuscularly [Fowler 2009]). Upon induction, animals were weighed and implanted with a passive integrated transponder tag (Avid Identification Systems, Norco, California, USA), and blood samples were collected. We transported raccoons to animal care facilities at the National Wildlife Research Center, Fort Collins, Colorado, where they were placed in individual pens (3×3×2.5 m) with a den box and enrichment structures.

Following release into pens, we collected the first fecal sample available to assess *B. procyonis* infection at the time of collection. For 16 of the 21 raccoons used in the trial, we presented one anthelmintic bait immediately after the first fecal collection. If fecal collection occurred on the same day as capture, the anthelmintic bait was the only source of food available during the first night in captivity. If the animal did not defecate within 24 h of collection, we provided a daily food ration (180 g, Mazuri Omnivore, PMI Nutrition International, Brentwood, Missouri, USA) until fecal collection occurred. Once feces were collected from these individuals, one anthelmintic bait was provided with the daily food ration, although the food ration was removed at the end of the day, leaving only the anthelmintic

bait overnight. We maintained this pattern of food and bait availability for five nights or until the bait was consumed; after five nights any uneaten portion of the bait was removed. In response to the reluctance of some of the first 16 raccoons used in the trial to consume an anthelmintic bait directly, we crumbled and mixed an anthelmintic bait with 155.9 g of moist cat food for the final five raccoons added to the captive population after these animals had been held in captivity up to 20 d. We removed any remaining cat food after 24 h and evaluated how much of the cat food/bait mixture had been consumed.

To determine whether bait consumption effectively cleared *B. procyonis* infections, we performed fecal flotation (Foreyt 2001) on samples collected before treatment (described earlier) and 7, 14, 28, 60, and 90 d after presentation of an anthelmintic bait. Samples were individually collected and stored frozen until they were screened for *B. procyonis* eggs. Because freezing negatively affects the ability of parasite elements to float (Foreyt 1986; Van Wyk and Van Wyk 2002), we also examined the sediment that remained on the bottom of the tube for eggs following centrifugation. Additionally, we visually examined fecal samples collected 1–4 d after anthelmintic bait consumption for passed nematodes. If eggs were present on day 14 after bait consumption, we provided an additional bait mixed in cat food, as described above, within 6 wk of the initial presentation of the anthelmintic bait. If infection persisted through two doses with anthelmintic baits, raccoons were dewormed with liquid pyrantel pamoate (20 mg/kg) mixed in cat food. Activities associated with these captive trials were approved by the USDA National Wildlife Research Center Institutional Animal Care and Use Committee under protocol QA-2111.

Field trials

To evaluate palatability of anthelmintic baits, we conducted field trials using remote cameras to monitor patterns of bait consumption for anthelmintic baits relative to standard fishmeal polymer baits. Trials were conducted in three disjunct mature hardwood forest patches in Tippecanoe County, Indiana, between 19 September and 7 November 2013. Each forest patch was adjacent to row crop agriculture with exurban development nearby and had water available in ponds or creeks. For each trial, we tethered either an anthelmintic or standard bait to a tree so that animal interactions with the bait could be captured by a remote camera. Specifically, we looped a 1.6-mm wire rope through the empty chamber of the bait and attached the wire rope to the tree so that the bait was 15 cm above the forest floor (Fig. 1). We then affixed a motion-activated, camera (PC900, RECONYX Inc., Holmen, Wisconsin, USA) to an adjacent tree with the camera programmed to capture images continuously through the duration of the detected motion (approximately two images per second). Additionally, we programmed cameras to capture a time-lapse image every 30 min; this allowed us to identify the time the bait was consumed if the camera failed to detect the bait removal. We established camera stations in a grid with 100-m spacing throughout the forest patches, alternating bait type (anthelmintic versus standard) between adjacent stations. Bait densities of 100/km^2 were realistic for management applications, similar to densities in raccoon rabies ORV baiting (75 or 150 baits/km^2; Slate et al. 2009). We maintained six to nine cameras at

FIGURE 1. Experimental setup used to monitor via remote camera in situ bait consumption for standard fish meal polymer baits and experimental anthelmintic baits (220 mg pyrantel pamoate [base] added to standard bait mixture of fishmeal, fish oil, and binding agent) in three disjunct forest patches in Tippecanoe County, Indiana, USA, in 2013.

a time, moving camera stations through the grid after each trial. Trials began at sunset on day 1 for all cameras deployed simultaneously. We used imagery and associated time stamps to identify the outcome of bait contact (consume or ignore), the species contacting the bait, and the time the interactions occurred. We calculated Kaplan-Meier estimates to evaluate differences between bait types in the time to consumption and conducted a test of equal proportions to evaluated whether the proportion of baits consumed by raccoons as opposed to nontarget species differed by bait type. Characterizing the outcome of raccoon visits to a bait as either consume (1) or ignore (0), we used logistic regression to evaluate the influence of bait type on the probability that a raccoon consumed the bait. All of these analyses were conducted in R (R Development Core Team 2014). All in situ assessments of bait consumption conformed to Purdue University Animal Care and Use Committee policies (Protocol 1211000761).

RESULTS

Captive trials (Table 1)

Of the 21 raccoons in the captive trial, 16 had patent infections, actively shedding *B. procyonis* eggs at the time of capture. Of the 16 raccoons that were presented an anthelmintic bait directly, the willingness to consume a bait varied among individuals, with five consuming the entire bait, four consuming >10 g but less than the entire bait, five consuming a small portion of the bait (1.26–3.78 g), and two consuming <1 g. Of the subsequent five raccoons that were presented a crumbled bait mixed with moist cat food, three consumed the entire mixture, whereas two consumed only a small portion and therefore received a reduced pyrantel dose.

Of the 16 infected raccoons, 10 consumed >10 g of an anthelmintic bait (directly $n=7$, mixed with cat food $n=3$). Of these 10 individuals, seven were no longer shedding eggs when re-evaluated 7 d postbait consumption, nor were eggs detected during the 90-d monitoring period. Two of the three raccoons with persistent infections were presented with a second anthelmintic bait. Of these two raccoons, *B. procyonis* infection was cleared in one, as indicated by lack of eggs in the feces. The second continued to shed eggs after the consumption of a second bait; a third treatment with liquid pyrantel pamoate also failed to eliminate *B. procyonis*. Of the six infected raccoons that consumed only a portion of the first bait, four were no longer shedding eggs when evaluated at 14 d. For the remaining two raccoons, infections were cleared with the complete consumption of a second anthelmintic bait mixed in cat food. Two of the four raccoons that ceased shedding eggs 14 d after partial bait consumption resumed egg shedding later in the trial (eggs detected at 28 d for one raccoon and 60 and 90 d for the second); however, these individuals were not treated a second time within the context of this study.

Of the 16 raccoons collected with patent infections, adult *B. procyonis* were observed in feces of 13 raccoons shortly after initial bait consumption (8/10 with complete bait consumption; 5/6 with partial bait consumption). Of the five individuals for which infection persisted after the consumption of either a complete ($n=3$) or partial ($n=2$) bait, nematodes were observed in the feces of three. No nematodes were detected in the feces of the five raccoons that were not shedding eggs at the time of capture.

Field trials

We conducted 70 bait trials (36 anthelmintic, 34 standard baits) from which the time to bait consumption could be determined for 60 baits (32 anthelmintic, 28 standard baits; four anthelmintic and two standard baits were still present when cameras were serviced and therefore censored for survival analysis), and the species consuming the bait was identified for 51 trials (27 anthelmintic, 24 standard baits; cameras failed to detect motion associated with bait removal of five anthelmintic and eight standard baits).

TABLE 1. Progress of *Baylisascaris procyonis* infection in naturally infected male (M) and female (F) raccoons (*Procyon lotor*) after exposure to fishmeal polymer baits intended to deliver 220 mg of pyrantel pamoate (base) with complete bait consumption.

Animal ID	Sex	Weight (kg)	Method[a]	Bait consumed (g)[b]	Realized dose rate (mg/kg)	Nematodes present[c]	Pretreatment[d]	Day 7[e]	Day 14	Day 28	Day 60	Day 90
Infected raccoons—single dose												
97-288	F	5.2	Direct	1.26	3.55	+	+	−	−	−	+	+
96-355	F	9.0	Direct	15.82	25.78	+	+	−	−	−	−	−
94-001	M	8.8	Direct	11.39	18.98	+	+	−	−	−	−	−
93-811	M	7.4	Direct	3.78	7.49	+	+	+	−	+	−	−
92-346	M	6.6	Direct	11.79	26.20	+	+	−	−	−	−	−
87-334	M	5.5	Direct	11.11	29.63	+	+	−	−	−	−	−
80-075	F	6.6	Direct	15.67	34.82	+	+	−	−	−	−	−
35-788	F	7.0	Direct	2.18	4.57	+	+	−	−	−	−	−
16-662	F	7.6	Cat food	Partial	—	−	+	−	−	−	−	−
22-893	M	10.0	Cat food	15	22.00	+	+	−	−	−	−	−
89-894	F	5.2	Cat food	15	42.31	+	+	−	−	−	−	−
Infected raccoons—multiple doses												
21-374	M	6.0	Direct	0.15	0.37	+	+	+	+	NA[f]	NA	NA
Dose 2			Cat food	15	36.67	+	NA	−	−	−	−	−
27-357	F	5.0	Direct	16.35	47.96	−	+	+	+	NA	NA	NA
Dose 2			Cat food	15	44.00	+	NA	−	−	−	−	−
31-830	F	5.0	Direct	1.94	5.69	+	+	+	+	NA	NA	NA
Dose 2			Cat food	15	44.00	−	NA	−	−	−	−	−
94-855	M	6.5	Direct	10.45	23.58	+	+	+	+	NA	NA	NA
Dose 2			Cat food	15	33.85	−	NA	+	+	NA	NA	NA
Dose 3			Liquid		20	+	NA	−	−	+	+	+
22-811	M	6.2	Cat food	15	35.48	−	+	+	+	NA	NA	NA
Dose 2			Liquid		20	+	NA	−	−	−	−	−
Uninfected raccoons												
09-057	M	7.20	Direct	0.56	1.14	−	−	−	−	−	−	−
15-821	F	6.00	Direct	15.52	37.94	−	−	−	−	−	−	−
27-776	M	9.20	Direct	1.55	2.47	−	−	−	−	−	−	−
75-380	M	7.00	Direct	15.58	32.64	−	−	−	−	−	−	−
01-256	F	6.40	Cat food	0	0.00	−	−	−	−	−	−	−

[a] Baits were either presented directly to raccoons (Direct) or were crumbled and mixed with moist cat food (Cat food). If raccoons remained infected after bait consumption, they were treated with 20 mg/kg pyrantel pamoate delivered in moist cat food (Liquid).

[b] Actual amount of bait consumed relative to a nominal 15-g bait intended to deliver 220 mg of pyrantel pamoate (base).

[c] Presence (+) or absence (−) of adult *B. procyonis* nematodes observed among raccoon feces collected 1–4 d after bait presentation.

[d] Presence (+) of *B. procyonis* eggs in raccoon feces at the time in which wild-caught raccoons were brought into captivity.

[e] Raccoon feces were evaluated for the presence (+) of *B. procyonis* eggs 7, 14, 28, 60, and 90 d after bait exposure. If eggs were present 14 d after bait exposure, raccoons received a follow-up treatment.

[f] NA = not applicable.

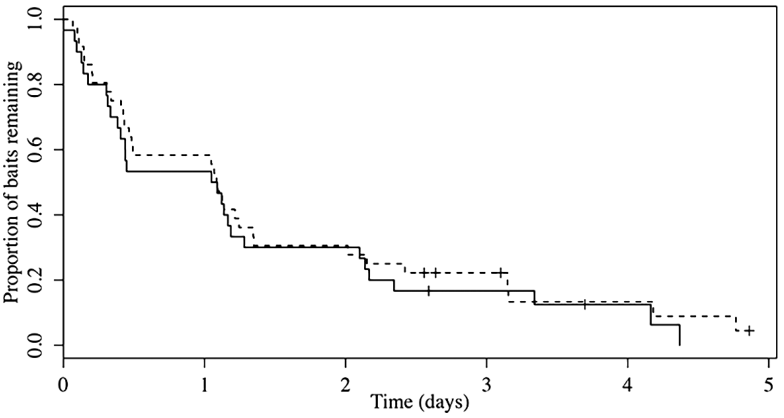

FIGURE 2. Proportion of standard (solid line) fishmeal polymer baits and experimental anthelmintic (dashed line) baits (220 mg pyrantel pamoate [base] added to standard bait mixture of fishmeal, fish oil, and binding agent) remaining during bait consumption trials conducted in three disjunct forest patches in Tippecanoe County, Indiana, USA, with bait consumption monitored by remote cameras. Baits that remained unconsumed at the conclusion of the trial period were right censored for analysis and are indicated with +.

Both bait types were consumed rapidly in situ (Fig. 2) with 44% (15/36 anthelmintic; 14/30 standard) consumed in the first night, 70% by the end of the second night (25/36 anthelmintic; 21/30 standard), and 80% by the end of the third (28/36 anthelmintic; 25/30 standard). There was no difference in the rate of bait consumption between the two bait types ($\chi^2=0.40$, df=1, $P=0.50$). Of the trials in which we could identify the species consuming the bait, raccoons consumed 10 of 27 (37%) anthelmintic baits and 12 of 24 (50%) standard baits. Differences in the proportion of baits consumed by raccoons were not statistically significant ($\chi^2=0.42$, df=1, $P=0.52$). Raccoons approached anthelmintic baits on 39 occasions and consumed 10 of these baits, whereas raccoons contacted standard baits on 21 occasions and consumed 12 baits. Bait type was a significant predictor of the outcome of bait contact by raccoons ($P=0.02$, odds ratio=3.89 for standard vs. anthelmintic), indicating that, upon encounter, raccoons were more willing to consume standard baits than anthelmintic baits. Virginia opossums (*Didelphis virginiana*) were the only nontarget species that consumed baits (consuming 17 anthelmintic in 24 encounters and 12 standard in 12 encounters). We observed bait contact by flying squirrels (*Glaucomys volans*) and white-footed mice on multiple occasions; however, these small-bodied animals were unable to remove baits from tethers.

DISCUSSION

Anthelmintic fishmeal polymer baits were moderately effective for eliminating *B. procyonis* infections in naturally infected raccoons. Infections were eliminated in nine of 12 raccoons when individuals consumed >10 g (of 15 g) of a bait, resulting in realized dose rates of 18.9–48.0 mg/kg. Of those individuals with persistent infections, the presence of adult *B. procyonis* in feces suggests that parasite numbers were reduced, although not eliminated, with bait consumption. Bauer and Gey (1995) found that one dose of pyrantel delivered in food at 20 mg/kg cleared 100% of *B. procyonis* infection in captive raccoons. Additional work is needed to determine why infection persisted among some individuals after bait consumption. Tetracycline is often incorporated into fishmeal polymer ORV baits as a biomarker. High-performance liquid chromatography analyses of bait contents

have demonstrated that baits deliver a lower effective dose of tetracycline than intended because of degradation during the bait manufacturing process and portions of the dose being functionally unavailable by binding with polymer in the bait formulation (Johnston et al. 2005). Similar processes may have limited the effective dose of pyrantel delivered in baits and contributed to the failure to deworm three of 12 individuals. However, therapeutic use of anthelmintics often requires multiple doses to clear infection (Overgaauw 1997), and it is recommended that raccoons taken into captivity receive multiple treatments to ensure complete elimination of *B. procyonis* (Kazacos 2001). Management applications of anthelmintic baits for *B. procyonis* mitigation will require repeated bait distribution because of the potential for raccoons to be reinfected from environmental reservoirs (Hegglin and Deplazes 2008; Page et al. 2011; Smyser et al. 2013). Therefore, should initial bait contact result in reduction, but not elimination, of *B. procyonis* infections, raccoons likely would receive additional treatments with subsequent bait distribution.

The range of realized dosages in our study illustrates one of the challenges of using medicinal baits to manage disease among free-ranging wildlife. Our intent in anthelmintic bait development was to deliver a minimum pyrantel dose of 20 mg/kg. A failure of some individuals to consume an entire bait, thereby receiving a dose below recommended therapeutic levels, may have contributed to the persistence of infection in some individuals. Furthermore, pyrantel is intended to be consumed as a single dose. However, some raccoons consumed one bait over multiple days (S.R.J. unpubl. data); it is possible that these individuals did not consume a sufficient amount of bait at one time to obtain a therapeutic dose. Images collected during our in situ trials provided no evidence of similar behavior among free-ranging raccoons; among the 22 anthelmintic and standard baits consumed by raccoons, we did not observe raccoons discarding a partially eaten bait. While this behavior may occur in the wild, the reluctance to consume baits and the consumption of only portions of baits observed in captive trials were likely exacerbated by the stress associated with transfer from the wild to the captive facility immediately before the initiation of bait trials.

We could not determine whether the failure to clear *B. procyonis* infection from some raccoons may have been attributable to the presence of nematodes with genotypes resistant to pyrantel. Given that free-ranging raccoons would not have been treated previously with pyrantel, we would not expect anthelmintic-resistant *B. procyonis* to have a selective advantage over susceptible nematodes in the general population. Thus, this hypothesis for the cause of persistent infections seems unlikely. However, anthelmintic resistance among gastrointestinal helminths of domestic animals is a major challenge in veterinary medicine (Kaplan 2004; Wolstenholme et al. 2004), and similar challenges could arise with the broad-scale distribution of anthelmintic baits for *B. procyonis* control. Among domestic animals, emergence of anthelmintic resistance appears to be associated with a lack of refugia for anthelmintic-susceptible parasites and administration of dosages below therapeutic levels (Falzon et al. 2014). Anthelmintic bait distribution for *B. procyonis* control would maintain abundant refugia for susceptible nematodes. Even if broadly adopted, we would expect baiting to be restricted to urban and suburban landscapes, where the risk of human exposure is greatest. Therefore, *B. procyonis* infecting raccoons in outlying rural habitats would represent a large refugium with the potential to continually introduce susceptible parasites via raccoon immigration. Moreover, egg and larval stages in the environment or paratenic hosts are unaffected by raccoon

treatment and serve as refugia. Finally, baits were developed to deliver a therapeutic dose for raccoons in the 95th percentile by weight. In management applications, the density of distributed baits would exceed raccoon densities, thus creating potential for raccoons to consume multiple baits, and would reduce the risk of under-dosing large raccoons. Collectively, multiple factors should hinder the emergence of anthelmintic resistance in *B. procyonis*.

Despite consumption of anthelmintic and standard baits in situ at similar rates, we observed a consistent preference by raccoons for standard baits. A greater proportion of standard baits was consumed by raccoons (50% standard vs. 37% anthelmintic) and, when encountered in the landscape, raccoons consumed standard baits more frequently than anthelmintic baits (26% [10/39] anthelmintic vs. 57% [12/21] standard). In contrast, opossums seldom failed to consume a bait of either type once encountered (81% [29/36]). Of the seven encounters in which opossums failed to consume a bait, imagery suggested opossums attempted and failed to remove the bait from the tether on three occasions rather than simply ignoring the baits. In sum, nontarget opossums were less discriminating between bait types than sympatric raccoons. Bait consumption by opossums can limit bait availability for raccoons (Olson and Werner 1999; Olson et al. 2000; Smyser et al. 2010, 2013). Therefore, if raccoons are more reluctant to consume anthelmintic baits, then opossums, as less selective foragers, could have an even greater effect on reducing bait availability for raccoons relative to standard baits. Additional work is needed to evaluate whether pyrantel pamoate in anthelmintic baits can be masked by increasing fish oil content or introducing molasses or other flavor enhancers to improve palatability (Hanlon et al. 1989).

Initial trials of a mass-producible anthelmintic bait for the control of *B. procyonis* in free-ranging raccoons appear promising. The small batch of anthelmintic baits used in this trial cost $0.50/bait, a savings of $0.10/bait over the multiple-ingredient baits (fishmeal attractant, anthelmintic, marshmallow cream, paraffin wax) used previously in field trials (Page et al. 2011; Smyser et al. 2013). At mass production scales, the introduction of pyrantel pamoate to fishmeal polymer baits could add as little as $0.05 to the cost of standard baits. Furthermore, by integrating pyrantel pamoate into the fishmeal matrix, the chamber of the bait remains available to deliver vaccine, medicinal, or contraceptive products. Anthelmintic baits could be coupled with an oral rabies vaccine to manage two zoonotic diseases with one bait if the addition of pyrantel does not reduce vaccine efficacy or bait palatability (Roberts and Aubert 1995). Continued work is needed to develop appropriate strategies to mitigate the risk posed by *B. procyonis* to human health. With refinement in dose and palatability, we believe anthelmintic baits could contribute to this goal.

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