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## Gastrointestinal Helminths of Free-ranging Florida Panthers (*Puma concolor coryi*) and the Efficacy of the Current Anthelmintic Treatment Protocol

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**ABSTRACT:** Thirty-five Florida panthers (*Puma concolor coryi* [Bangs, 1899]) collected from six counties in southern Florida between 1978 and 2003 were examined at necropsy for gastrointestinal helminths. The panthers were placed into two groups: 1) treated with anthelmintics ( $n=17$ ), and 2) untreated ( $n=18$ ). Nine species of helminths (one trematode, six nematodes, and two cestodes) were identified in the untreated panthers. The most prevalent helminths were *Alaria marcianae* (LaRue, 1917) (100%), *Spirometra mansonioides* (Mueller, 1935) (91%), and *Ancylostoma pluriidentatum* (Alessandrini, 1905) (89%). *Ancylostoma caninum* (Ercolani, 1859) is reported from the Florida panther for the first time. The intensities of helminths with prevalences  $>10\%$  did not differ between untreated panthers collected in 1978–1983 and 1996–2003. Treated panthers had helminth faunas similar to those of untreated panthers. The current anthelmintic treatment being used reduced the intensity of both *A. marcianae* and *A. pluriidentatum* in panthers  $\leq 6$  mo posttreatment (PT); however, treated panthers between 6 and 9 mo PT, and  $>9$  mo PT were similar to untreated panthers. Treatment was less effective on *S. mansonioides* and *Taenia omissa* Lühe, 1910. Treated panthers had slightly lower intensities of *S. mansonioides* at  $\leq 6$  mo PT; however, between 6 and 9 mo PT and  $>9$  mo PT they had significantly higher intensities than untreated panthers. At all periods PT, the intensity of *T. omissa* for the treated panthers was similar to that of untreated panthers. We suggest that *Mesocestoides* sp. may not be present in the Florida panther population as reported earlier by Forrester et al. (1985), due to parasite misidentification by those authors.

**Key words:** Anthelmintic, Florida, Florida panther, helminths, parasites, *Puma concolor coryi*.

The Florida panther (*Puma concolor coryi* [Bangs, 1899]) was placed on the

endangered species list by the U.S. Department of Interior in 1967 (Federal Register - 48). Since the early 1980s the Florida Fish and Wildlife Conservation Commission (FWC) has been closely monitoring the health of the panther population. As the monitoring evolved, a protocol was set up to treat the adult panthers with anthelmintics. To date the efficacy of this anthelmintic treatment has not been evaluated, mainly due to the lack of a large-enough sample of treated and untreated panthers being examined for helminths. This study was conducted to evaluate the efficacy of the current anthelmintic protocol being utilized.

Between 1996 and 2003, 23 Florida panthers (FP) (11 females, 12 males) were collected from the following six counties in southern Florida: Collier,  $26^{\circ}10'N$ ,  $81^{\circ}30'W$  ( $n=17$ ); Glades,  $26^{\circ}55'N$ ,  $81^{\circ}15'W$  ( $n=1$ ); Hendry,  $26^{\circ}30'N$ ,  $81^{\circ}15'W$  ( $n=3$ ); Hillsborough,  $27^{\circ}45'N$ ,  $82^{\circ}10'W$  ( $n=1$ ); and Palm Beach,  $26^{\circ}45'N$ ,  $80^{\circ}30'W$  ( $n=1$ ). From 1988 to 1993, five FPs (all males) were collected from Collier ( $n=3$ ) and Hendry ( $n=2$ ) counties. Between 1978 and 1983, 7 FPs (three females, four males) were collected from Collier ( $n=6$ ) and Glades ( $n=1$ ) counties; data from these latter panthers were reported previously by Forrester et al. (1985).

The FPs collected were classified as: 1) untreated, not captured previously, and 2) treated, captured previously and treated with anthelmintics, and routinely recaptured every 2–3 yr for radio replacement and retreated with anthelmintics. Treated panthers were given injectable ivermectin

(Ivomec® 1%, Merial Limited, Iselin, New Jersey, USA; 0.2 mg/kg) and praziquantel (Droncit®, Bayer Health Care, Tarrytown, New York, USA; 5.0 mg/kg) subcutaneously. The treated panthers ranged from 10 mo to 19.5 yr old ( $\bar{x}$ =5.85 yr) and were treated from 1 to 10 times ( $\bar{x}$ =2.6) in their lifetime. Four of the treated FPs were also treated orally with pyrantel pamoate (Anthelban-V®, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, USA; 5 mg/kg) at 1–3 wks old in the den. The complete biomedical protocol for Florida panthers and Texas cougars (*Puma concolor*) captured in Florida was reported by Shindle et al. (2003).

Parasite screening techniques used were those described by Kinsella and Forrester (1972). Trematodes and cestodes were preserved in Roudabush's AFA, stained with either Ehrlich's hematoxylin or Semichon's acetic-carmin, and mounted in neutral Canada balsam. Nematodes were preserved in 70% ethanol with glycerin, mounted in lactophenol for identification, and then returned to the preservative.

A chi-square test was used to compare prevalences, depending on the normality of the data set, a *t*-test or Mann-Whitney rank sum test to compare intensities of the parasites, and a Spearman rank correlation was used to compare FP age to parasite intensities with the use of SigmaStat® for Windows (Version 2.03, SPSS, Inc., Chicago, Illinois, USA). Significance was accepted at  $P \leq 0.05$ . Treated panthers were arranged in ascending order according to the length of time (in months) since their last treatment (PT = posttreatment). Intensities for the untreated panthers were considered baseline intensity levels. Then the PT intensities for the treated panthers were compared to these baseline levels to determine the efficacy of the anthelmintic treatment. However, because of uncertainty about the correct identification of the cestodes in the 1978–83 sample year set (Forrester et al., 1985), these seven panthers were not used when

*Spirometra mansonoides* or *Taenia omissa* were analyzed.

Terminology used follows Bush et al. (1997). Helminth voucher specimens have been deposited in the U.S. National Parasite Collection (USNPC), Beltsville, Maryland, USA (accession numbers USNPC 94199-94202 and 94310-94315).

Eighteen untreated free-ranging FPs (six females, 12 males) and 17 anthelmintic-treated free-ranging FPs (six females, 11 males) were examined for gastrointestinal helminths. Ages ranged from 6 mo to 14 yr ( $\bar{x}$ =3.6 yr) for untreated panthers, and 10 mo to 19.5 yr ( $\bar{x}$ =5.85 yr) for treated panthers. Nine species of helminths (one trematode, six nematodes, and two cestodes) were identified from the 35 Florida panthers. The prevalences and intensities of helminths for untreated panthers are presented in Table 1. The most prevalent parasites were *Alaria marcianae*, *Ancylostoma pluridentatum*, *Spirometra mansonoides*, and *Taenia omissa*, and their intensities were compared between treated and untreated panthers.

There were no differences in intensities of either *A. marcianae* or *A. pluridentatum* between the 1978–83 and the 1996–2003 samples of untreated panthers ( $P=0.06$  and  $P=0.95$ , respectively). Only one panther from the 1988–1993 sample was untreated, and the intensities for both *A. marcianae* and *A. pluridentatum* were within the range and 1 SD of the mean intensities for these parasites in the 1996–2003 sample. Because there were no differences in intensities for the two most prevalent parasites for these three sets of sampling years, all untreated Florida panthers from the three sets of sampling years were combined for a single grouping for further statistical analysis. Consequently, we combined all the treated panthers from the three sets of sampling years into a single treated group.

Two of the treated panthers collected at 0.1 and 1.0 mo PT were free of intestinal helminths except for the presence of two

TABLE 1. Prevalences and intensities of gastrointestinal helminths in 18 untreated free-ranging Florida panthers collected between 1978 and 2003 from southern Florida.

Helminth species	Prevalence (%)	Intensity	
		Mean (SD) <sup>b</sup>	Range
Trematodes			
<i>Alaria marcianae</i> (LaRue, 1917) (SI) <sup>a</sup>	100	1629.7(2533.78)	93–9689
Cestodes <sup>c</sup>			
<i>Spirometra mansonoides</i> (Mueller, 1935) (SI)	91	9.6(11.68)	1–33
<i>Taenia omissa</i> Lühe, 1910 (SI)	64	12.6(10.20)	1–34
Nematodes			
<i>Ancylostoma pluridentatum</i> (Alessandrini, 1905) (SI)	89	234.3(260.14)	36–865
<i>Ancylostoma caninum</i> (Ercolani, 1859) (SI)	11	1.5(0.53)	1–2
<i>Molineus barbatus</i> Chandler, 1942 (SI)	11	11.1(4.84)	2–20
<i>Physaloptera rara</i> (ST, SI)	6	1.0	–
<i>Strongyloides</i> sp. (SI)	22	6.5(4.04)	1–16
<i>Toxocara mystax</i> (ST, SI)	6	2.0	–
<i>Toxocara</i> larvae (SI) <sup>d</sup>	39	5.1(10.20)	1–20

<sup>a</sup> Location in host: ST = stomach, SI = small intestine.

<sup>b</sup> SD = standard deviation.

<sup>c</sup> Cestode results based on 11 panthers.

<sup>d</sup> Most likely larvae of *T. mystax*.

*A. marcianae* and 14 *S. mansonoides*. *Ancylostoma pluridentatum* was present at 2 mo PT and *T. omissa* at 6 mo PT. Intensities and prevalences of helminths in the treated panthers are presented in Table 2.

Intensities of both *A. marcianae* and *A. pluridentatum* were significantly lower in treated panthers  $\leq 6$  mo PT ( $P=0.016$  and  $0.001$ , respectively); however, treated panthers between  $>6$  and  $\leq 9$  mo PT were similar to untreated panthers ( $P=0.65$  and  $0.23$ ), as were treated panthers  $>9$  mo PT ( $P=0.77$  and  $0.43$ ).

Treated panthers had slightly lower intensities of *S. mansonoides* at  $\leq 6$  mo PT; however, between  $>6$  and 9 mo PT and  $>9$  mo PT they had significantly higher intensities than untreated panthers ( $P=0.036$  and  $0.001$ , respectively). At all periods PT the intensity of *T. omissa* for the treated panthers was similar to that of untreated panthers ( $P=0.14$ – $0.76$ ).

There were no significant correlations between the age of untreated FPs and the intensities of any of the helminths ( $r_s=-0.333$  to  $0.296$ ;  $P=0.27$ – $0.72$ ). There was also no correlation between

time PT and intensities of helminths for treated FPs  $>6$  mo PT ( $r_s=-0.190$  to  $0.147$ ;  $P=0.50$ – $0.93$ ).

When comparing the helminths reported by Forrester et al. (1985) and the helminths from this study (yrs 1988–2003 sampling set), one obvious difference is the absence of *Mesocestoides* sp. in the current study. Forrester et al. (1985) reported that four of seven (57%) FPs examined were infected with *Mesocestoides* sp., having a mean intensity of 212 and a range of 1–833. The voucher specimens identified by Forrester et al. (1985) as *Mesocestoides* sp. and deposited in the USNPC (USNPC No. 78390, two slides) were examined and found to be *Spirometra*, not *Mesocestoides*. The *S. mansonoides* vouchers (USNPC No. 78391–78392) of Forrester et al. (1985) were examined to see if an error in labeling occurred before the vouchers were sent to the USNPC. Both were determined to be *S. mansonoides*. In addition, all vials and slide mounts of cestodes retained in the laboratory collection of those authors were examined, and none were found to be *Mesocestoides* sp. All cestodes that were

TABLE 2. A comparison of prevalences and intensities of gastrointestinal helminths in anthelmintic treated free-ranging Florida panthers collected between 1978 and 2003 from southern Florida.

Helminth species	Months posttreatment					
	≤6 (n=6)		>6–9 mo (n=5)		>9 (n=6)	
	Prevalence (%)	Intensity <sup>a</sup>	Prevalence (%)	Intensity	Prevalence (%)	Intensity
		Mean (SD) <sup>b</sup>		Mean (SD)		Mean (SD)
<b>Trematodes</b>						
<i>A. marcianae</i>	100	150.7(196.9)A <sup>c</sup>	100	776.4(1059)B	100	1301.9(549.6)B
<b>Cestodes</b>						
<i>S. mansonioides</i>	100	47.7(51.9)A	80	52.4(56.8)A,B	100	161.1(156)A,B
<i>T. omissa</i>	17	1.7(4.1)B	60	2.8(4.2)B	83	1.4(1.4)B
<b>Nematodes</b>						
<i>A. pluridentatum</i>	67	14.3(17.5)A	100	179.6(223.4)B	100	297.3(319.8)B
<i>A. caninum</i>	0	–	20	0.2(0.4)	0	–
<i>M. barbatus</i>	0	–	20	0.2(0.4)	17	0.6(1.5)
<i>P. rara</i>	0	–	0	–	0	–
<i>Strongyloides</i> sp.	17	1.0(2.4)	0	–	0	–
<i>T. mystax</i>	33	2.2(4.8)	40	5.0(10.6)	67	3.6(6.1)

<sup>a</sup> Calculations for mean intensities include intensities equal to zero in each group, as used for the statistical analysis.

<sup>b</sup> SD = Standard deviation.

<sup>c</sup> Similar letters indicate intensities that are statistically the same; intensities with a B are statistically the same as untreated Florida panthers.

not *T. omissa* were *S. mansonioides*. A small series of mounted specimens that were labeled “*Mesocostoides* sp.” were actually *S. mansonioides*. From our investigation of these cestodes, we believe that Forrester et al. (1985) incorrectly identified these cestodes as *Mesocostoides* sp. when they were actually *S. mansonioides*. Because of these findings and the fact that *Mesocostoides* sp. has not been found in over 35 FPs quantitatively and 10 FPs qualitatively analyzed for parasites, nor have eggs been detected in 65 panthers analyzed via fecal examination (E. C. Greiner, pers. comm.) since 1984, we cautiously suggest that *Mesocostoides* is not present in the Florida panther population. However, *Mesocostoides lineatus* (Goeze, 1782) has been reported from cougars (*P. concolor*) in northeastern Oregon (Rausch et al., 1983).

It appears that the current anthelmintic treatment protocol given the FPs is effective in reducing intensities of *A. marcianae*, *A. pluriidentatum*, and *S. mansonioides* up to 6 mo PT, but at >6 mo PT the intensities are similar to

those of untreated PFs. However, the intensities of *T. omissa* were similar between treated and untreated FPs at all periods PT. Because of small sample sizes, it would be difficult to infer accurately what effect the treatment might have on the helminths that occurred at lower prevalences. However, by 11 mo PT, except for *P. rara*, all of the helminths found in the untreated FPs were present in the treated FPs.

At the time of death most of the untreated FPs were in good nutritional condition, an indication that adult FPs were not adversely impacted by the intensities of parasite infection we found, without anthelmintic support. The rapid reinfection of the FPs is not surprising because the intermediate hosts for many of the parasites reported here are the common prey of the FP, and *Ancylostoma* spp. have a direct life cycle. Since the time between captures for each FP is approximately 2–3 yr and the time of reinfection to untreated levels PT is about 6 mo, the current anthelmintic protocol being utilized for adult FPs may not be useful.

However, the treatment of FP kittens may be of greater importance than treating adults. Dunbar et al. (1994) reported clinical signs of poor body condition, lethargy, and below normal hematological values in a 12–14-day-old FP kitten infected with *A. pluridentatum*. The kitten was treated orally with pyrantel pamoate at 20 mg/kg and ivermectin 0.20 mg/kg, and subsequently all clinical signs improved.

The statistical analysis presented here may be rather weak considering the small sample sizes and a few of the resulting *P* values being close to the *P*=0.05 level of significance.

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