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Source: Journal of Wildlife Diseases, 27(3) : 486-490

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-27.3.486>

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Hemoparasites of Raccoons (*Procyon lotor*) in Florida

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ABSTRACT: Four hemoparasite species (*Babesia lotori*, *Trypanosoma cruzi*, *Dirofilaria tenuis* and *Mansonella llewellyni*) were found in raccoons (*Procyon lotor*) collected from 1972 to 1974 in Duval ($n = 14$) and Collier ($n = 170$) counties, Florida (USA). *Trypanosoma cruzi* was found in thin blood smears from one raccoon at each locality. The prevalence of *B. lotori* was 79% and 80% in samples taken in December 1973 in Collier and Duval counties, respectively. No patent infections by *B. lotori* were detected in raccoons collected in Collier County in December 1972, but 42% of the raccoons examined in September 1973 were infected. In Collier County there were no significant differences in the prevalence of *B. lotori* by host sex or age. In Duval County, overall *D. tenuis* prevalence was 7%, whereas that of *M. llewellyni* was 14%; the latter species was not found in Collier County. Adult raccoons had a significantly greater prevalence of *D. tenuis* (32%) than did subadults and juveniles (7%), and male raccoons showed a significantly greater prevalence (51%) than did females (8%).

Key words: Raccoons, *Procyon lotor*, hemoparasites, *Babesia lotori*, *Trypanosoma cruzi*, *Dirofilaria tenuis*, *Mansonella llewellyni*, prevalence, survey.

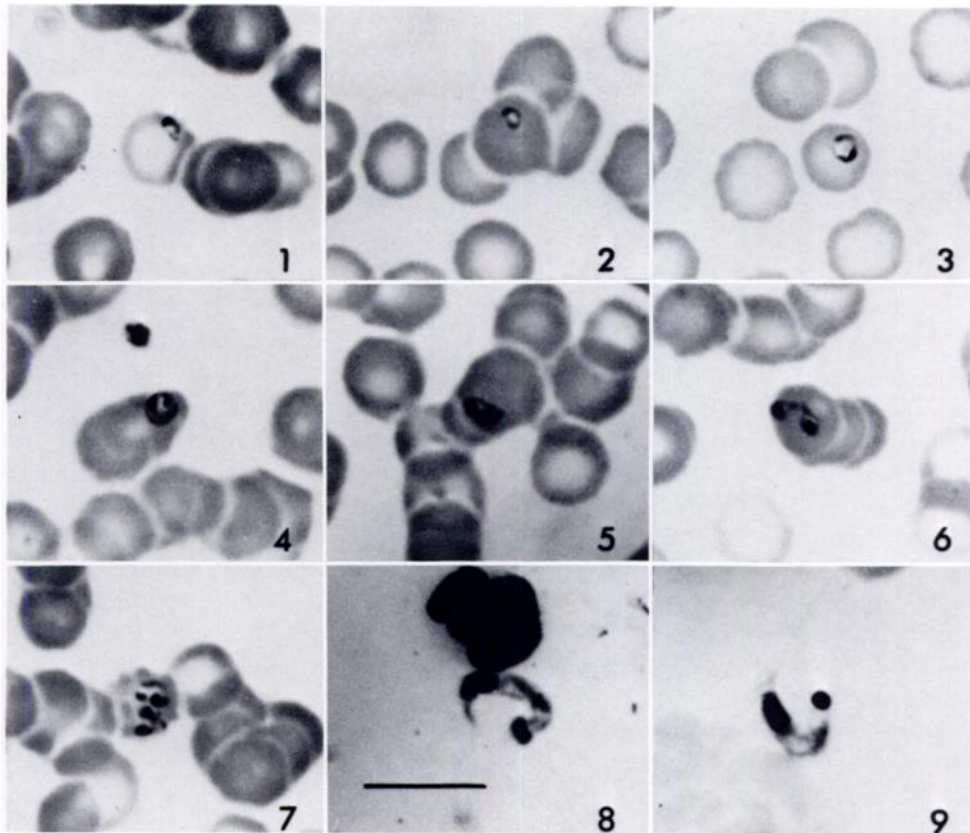
Interest in the blood parasites of raccoons in Florida has been limited to the possible dissemination of parasites through translocation of raccoons to other geographic areas (Schaffer et al., 1978) and to the potential of being reservoirs for zoonotic disease pathogens such as *Trypanosoma cruzi* (Beard et al., 1988) and *Dirofilaria tenuis* (Isaza and Courtney, 1988). Bigler et al. (1975) described the importance of raccoons as reservoirs of zoonotic diseases and as indicators of environmental health relative to pollutants in the southeastern United States.

While sampling populations of raccoons in northern and southern Florida in 1972 to 1974, thin blood smears were prepared from 184 individuals. These have been examined for hemoparasites and the results are the basis for this paper.

The raccoon sample from southern Florida was collected on Marco Island, Collier County (25°57'N, 81°42'W), and was comprised of 95 males and 75 females. Sixty-nine raccoons were obtained in December 1972, 24 in September 1973, and 77 in December 1973. Three age groups were represented, i.e., juveniles, subadults and adults, identified by skull suture closure, cementum layers and wear of teeth, eye lens weight, and reproductive characteristics (Bigler et al., 1981). The ratios of adults:subadults:juveniles for the three sampling periods were as follows: December 1972, 56:32:12; September 1973, 71:8:21; and December 1973, 70:12:18. Sex ratios (males:females) of the samples were, respectively, 1.23:1, 2.00:1, and 1.14:1. Adult males were 2.3-6.1 kg in weight, and 57-69 cm in total length; adult females were 2.1-5.6 kg and 63-75 cm; subadults were 1.1-4.9 kg and 51-75 cm (males); 1.1-3.9 kg and 51-73 cm (females); juveniles were 0.9-3.3 kg and 46-72 cm (males); 0.9-2.0 kg and 50-58 cm (females).

The small sample from northern Florida was collected at the Jacksonville Naval Air Station, Duval County (30°15'N, 81°47'W) in November 1973 ($n = 11$), May 1974 ($n = 1$), and December 1974 ($n = 2$), and was comprised of five adults (two males, three females; 3.4-4.2 kg), five subadults (two males, three females; 2.5-4.0 kg), three juveniles (one male, two females; 1.6-2.6 kg), and one of undetermined age and sex.

Thin blood films were prepared from undiluted cardiac blood in daytime immediately following euthanasia, fixed in absolute methanol, and stained with Giemsa by standard technique. There was no evident deterioration of stain quality when slides were examined following storage for 16 to 17 years. Slides were searched for microfilariae at 100 \times , for hemogregarines



FIGURES 1-7. *Babesia lotori* in erythrocytes of a raccoon from Marco Island, Florida. (1, 2) Uninucleate "ring" forms. (3, 4) Binucleate, spherical stages. (5, 6) Paired pyriform parasites. (7) Double infection of erythrocyte by paired pyriform stages. FIGURES 8, 9. Trypomastigotes of *Trypanosoma cruzi* in a cardiac blood film from a raccoon collected in Duval County, Florida. Host cells lysed in vicinity of trypanosome. Apparent division of kinetoplast in Figure 8 may be artifactual. Giemsa stain. Bar = 10 μ m.

and trypanosomes at 400 \times , and at 1,000 \times under oil immersion for piroplasms. Photomicrographs were taken at 1,000 \times . Data were analyzed by the Relfex Program (Borland International, Scotts Valley, California 95066, USA) for IBM compatible computers, and prevalences were compared by chi-square and the Fisher exact test, with significance accepted at $P \leq 0.05$. Representative blood films have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705, USA; Accession Numbers 81432-81435).

Four hemoparasite species were found in the raccoons: *Babesia lotori* (Figs. 1-7), *Trypanosoma cruzi* (Figs. 8, 9), *Dirofilaria tenuis*, and *Mansonella llewellyni* (Table 1). *Mansonella llewellyni* was detected

only at the northern locality, and the remaining three species were present at both sites.

The large sample of raccoons from southern Florida permitted examination of prevalence by host age, sex, and date of collection (Table 2). The overall prevalence of *B. lotori* was 41%, with no significant difference between males (44%) and females (35%). When the December 1972 sample, in which *B. lotori* was not detected, is excluded, the prevalence of patent infections between sexes in September and December 1973 was substantially higher than the overall comparison, 74% in males and 66% in females. Again, there were no differences in prevalence between sexes. No differences in prevalence were

TABLE 1. Prevalence of hemoparasites detected on thin blood films of raccoons from Marco Island, Collier County, and Jacksonville Naval Air Station, Duval County, Florida, 1972-1974.

Locality	Date	Number examined	Prevalence (%) of			
			<i>Babesia lotori</i>	<i>Trypanosoma cruzi</i>	<i>Dirofilaria tenuis</i>	<i>Mansonella llewellyni</i>
Collier County	12/72	69	0	1	16	0
	09/73	24	42	0	38	0
	12/73	77	79	0	26	0
Duval County	73-74	14	79	7	7	14

detected among adults (70%), juveniles (63%), and subadults (64%). The prevalence of *B. lotori* in the northern Florida samples taken in November 1973 was similar (82%) to that found in the total December 1973 sample (79%) from southern Florida (Table 1). Overall prevalence among all age groups from southern Florida was significantly higher ($P = 0.001$) in December (79%) than in September 1973 (42%).

The prevalence of *Dirofilaria tenuis* was significantly higher overall ($P = <0.01$) among adult male raccoons (31 of 61 infected, 51%) than among females (four of 49 infected, 8%), and this pattern occurred in each of the three sampling periods (Table 2). The prevalence of *D. tenuis* among adults did not differ among these samples, but significant differences were present overall among age groups ($P = <0.01$). Juveniles and subadults together showed significantly lower prevalence (7%, $P =$

<0.001) than did adults (32%). Infected juveniles were found only in September 1973. In the sample from northern Florida, *M. llewellyni* infections were present only in adults and subadults.

Trypanosoma cruzi, the causative agent of Chagas' Disease in humans, has been identified in raccoons throughout much of their range in the southern and eastern United States (Walton et al., 1958; McKeever et al., 1958; Olsen et al., 1964). Specific prevalence records from Florida were provided by Schaffer et al. (1978) for Glades (10%, $n = 10$) and Hillsborough counties (15%, $n = 20$). Irons (1971) reported serological evidence of *T. cruzi* in 2% of 144 raccoons collected in Alachua, Levy, and Hillsborough counties. Detection of *T. cruzi* on thin blood films is an inefficient survey method, yet single raccoons from Duval and Collier counties were positive for trypomastigotes (Figs. 8, 9). It is likely that raccoons throughout Florida

TABLE 2. Prevalence of *Babesia lotori* and *Dirofilaria tenuis* in Marco Island raccoons by age and sex of host in 1972 and 1973.

Age group and sex	Number examined			Prevalence (%)					
				<i>Babesia lotori</i>			<i>Dirofilaria tenuis</i>		
	12/72	09/73	12/73	12/72	09/73	12/73	12/72	09/73	12/73
All adults	39	17	54	0	41	80	26	29	37
Males	23	10	28		50	86	39	50	61
Females	16	7	26		29	73	6	0	12
All subadults	22	2	9	0	0	78	5	50	0
Males	10	2	2			50	10	50	0
Females	12	0	7			86	0	0	0
All juveniles	8	5	14	0	60	79	0	60	0
Males	5	4	11		75	82	0	75	0
Females	3	1	3		0	67	0	0	0

are infected with *T. cruzi*. Human infections in the southeastern United States have not been reported, but the potential importance of this zoonosis was demonstrated by Beard et al. (1988) when an isolate was obtained from a specimen of *Triatoma sanguisuga* collected in Gainesville, Alachua County and was the same zymodeme as one of the strains known to cause Chagas' Disease in humans in South America (Miles, 1983). While no studies have demonstrated that *T. cruzi* strains from Florida produce amastigotes in human cells either in vivo or in tissue culture, the demonstration of pathogenicity for a given host as a taxonomic character is secondary, in our opinion, to morphology (which includes biochemical characters such as the zymotype) for the definition and identification of a species. The strain isolated by Beard et al. (1988) justifies the appellation of *T. cruzi* rather than *T. cruzi*-like for these trypanosomes in raccoons from Florida.

The *Babesia* sp. parasitizing North American raccoons was designated *B. procyonis* by most authors until Anderson et al. (1981) described *B. lotori*, justifying taxonomic distinction on the basis of the smaller size of the parasite, its presence in a carnivore of a different family (Procyonidae) from that of the type host of *Piroplasma* (= *Babesia*) *procyoni* which was described from *Nyctereutes procyonoides*, the Ussurian raccoon dog (Canidae). Endemicity on a different continent was cited also in support of the species designation. *Babesia lotori* was reported as *B. procyonis* in Glades and Hillsborough counties in respective prevalences of 40% and 35% by Schaffer et al. (1978). Although not detected on thin blood films, chronic piroplasm infections may have been present in our December 1972 sample. In the study by Anderson et al. (1981), nine apparently negative raccoons became positive following splenectomy. The high prevalence of patent infections in the Collier County raccoons in December 1973 (79%), with several distinct forms (uni-

nucleate "rings," binucleate spherical forms, and paired pyriforms) present in the erythrocytes (Figs. 1-7), and the comparable prevalence of patent infections found in the northern Florida sample taken in November 1973 (80%) suggest that those samples were obtained immediately following a seasonal transmission period. This probably took place, at least in southern Florida, between 13-16 September (10 of 24 positive, 42%) and 3 to 6 December 1973 (61 of 77 positive, 79%). Transmission during this period possibly resulted from greater activity by infected tick vectors in response to a change in meteorological factors. Meteorological records for Naples (U.S. Department of Commerce, 1972-1973) indicated that precipitation declined sharply, by 93% in October to November 1973, 3.8 cm total, in contrast to the August to September total, 54.4 cm. In the preceding year, 1972, there was a less severe decrease in rainfall, 32%, from 40.9 cm, in August to September to 13.2 in October to November, which perhaps delayed transmission until sometime following the sample collection in December 1972.

Two other hemoparasites reported by Schaffer et al. (1978), *Hepatozoon procyonis* and *Haemobartonella procyoni*, were not detected in the raccoons examined by us, despite similar examination procedures.

Both filarial nematodes have been reported previously from raccoons in Florida. *Mansonella llewellyni* was found in one of 20 raccoons from Hillsborough County (Schaffer, 1979), and in two of four from Duval County (Forrester, 1991). This species was formerly known as *Dipetalonema llewellyni*, but is now considered to belong to *Mansonella* (Eberhard and Orihel, 1984). The extreme slenderness of the microfilariae of *M. llewellyni* distinguished it readily from *Dirofilaria tenuis*. Isaza and Courtney (1988) found a higher prevalence of *D. tenuis* in raccoons from Collier and Brevard counties in southern Florida, 45% and 21%, respectively, in

comparison to those examined from Alachua County (6%) in northern Florida. These data might support a relationship to the greater prevalence of *D. tenuis* in humans in southern Florida in comparison to other geographic areas. The presence of filarial infections in higher prevalence among older cohorts of the population in comparison to the younger is a commonly observed distributional pattern. The consistently higher prevalence of *D. tenuis* among male raccoons to that seen in females is difficult to explain, unless some behavioral difference between sexes results in more frequent exposure of the males to the mosquito vectors.

We wish to thank G. L. Hoff and W. J. Bigler for obtaining the blood films reported upon here, and P. P. Humphrey for her technical assistance in their preparation. We appreciate the help of M. D. Young, C. H. Courtney, and E. C. Greiner who reviewed the manuscript. Florida Agricultural Experiment Stations Journal Series No. R-01237.

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Received for publication 12 December 1990.