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Experimental Vectors of *Haemoproteus meleagridis* Levine from Wild Turkeys in Florida

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Haemoproteus meleagridis Levine occurs in wild turkeys (*Meleagris gallopavo* L.) throughout most of their range in North America (Greiner and Forrester, 1980, J. Parasitol. 66: 652–658). Its prevalence varies from 5% in Pennsylvania (Kozicky, 1948, J. Wildl. Manage. 12: 263–266) to 87% in southern Florida (Forrester et al., 1974, J. Protozool. 21: 494–497). Little is known about its life cycle. Attempts to transmit the infection by inoculation of blood and tissue homogenates have not been successful (Bierer et al., 1959, J. Am. Vet. Med. Assoc. 135: 181–182).

Several other avian species of *Haemoproteus* are transmitted by biting flies, viz. hippoboscids or ceratopogonids belonging to the genus *Culicoides* (Levine and Campbell, 1971, J. Protozool. 18: 475–484). Hippoboscid flies have been recorded rarely from wild turkeys collected in Alabama, Arkansas, Mississippi and West Virginia (Kellogg et al., 1969, J. Med. Entomol. 6: 329–330) and have never been found on wild turkeys collected in Florida (Forrester, unpubl. data). It is unlikely that they could maintain the high prevalences of the parasite found in the southeastern United States.

In May 1982, a study was initiated on the epidemiology of *H. meleagris* with the assumption that the most likely vectors were biting flies of the genus *Culicoides*. This note reports the potential vectors found and describes the first experimental transmission of the parasite.

Two-wk-old, broad-breasted white turkey poults were exposed to arthropod vectors in wooden cages screened with 1.3 cm wire mesh to monitor the natural transmission of *H. meleagridis* and to provide a source of infected birds. The cages were placed in a mixed deciduous forest inhabited by wild turkeys at Paynes Prairie State Preserve, 8 km SSE of Gainesville, Florida. Forrester et al. (1974, op. cit.) found that the parasite had a prevalence of 69% in wild turkeys from the same region. Every 2 wk, the sentinel poults were exchanged for a new group of 2-wk-old birds that had been reared in *Culicoides*-proof facilities. The exposed poults were moved to *Culicoides*-proof rooms and held for 4 wk. Blood smears from each bird were made three times a week from a leg vein. Infections were diagnosed by the examination of approximately 10,000 red blood cells per slide. All birds were fed and watered ab libitum. Sentinel poults that became infected were used as bait birds in a Bennett trap (Bennett, 1960, Can. J. Zool. 38: 377–389) for the collection of species of *Culicoides* at the sentinel sites. The trap was operated at dusk in the forest canopy where many ornithophilic species are most active (Tanner and Turner, 1974, Mosq. News 34: 66–70).

Engorged flies were kept at room temperature in half-pint cardboard cartons to allow sufficient time for development of the parasite and supplied with cotton pads moistened with a solution of 5% sucrose. After 1 wk the flies were identified and subsequently dissected in a drop of 0.85% saline. The midgut and salivary glands were removed from each fly and examined at 400× for oocysts and sporozoites. Infected salivary glands were drawn into the needle of a syringe and injected intraperitoneally (i.p.) or intravenously (i.v.) into uninfected poults. These birds were bled as described earlier for at least 4 wk.

Between May and December 1982, eight species of engorged *Culicoides* were captured in Bennett traps baited with infected turkeys (Table 1). Three species, i.e., *C. edeni*, *C. hinmani* and *C. arboricola*, were capable of supporting complete development of the parasite and had mature oocysts on the outer wall of their midguts and sporozoites present in their salivary glands by 6–10 days after engorging on an infected turkey (Fig. 1). Oocysts formed but did not complete their development by 4–10 days in *C. nanus*, *C. baueri* and *C. paraen-*

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TABLE 1. Susceptibility of wild *Culicoides* to *Haemoproteus meleagridis*.

Species of *Culicoides*[b]	Development of parasite[a]		
	C	P	N
C. edeni Wirth and Blanton	26/43 (60%)		
C. hinmani Khalaf	7/55 (13%)		
C. arboricola Root and Hoffman	3/16 (19%)		
C. nanus Root and Hoffman		7/21 (33%)	
C. paraensis (Goeldi)		1/4 (25%)	
C. baueri Hoffman		1/22 (5%)	
C. crepuscularis Malloch			1/1
C. haematopotus Malloch			2/2

[a] Fractions represent number of flies in which complete (i.e., invasion of salivary glands by sporozoites) (C), partial (i.e., development of degenerate oocysts) (P) or no development (N) occurred/total number of flies examined.

[b] Representative specimens deposited in the Florida State Collection of Arthropods, Florida Dept. of Agriculture and Consumer Services, Gainesville, Florida 32602, USA.

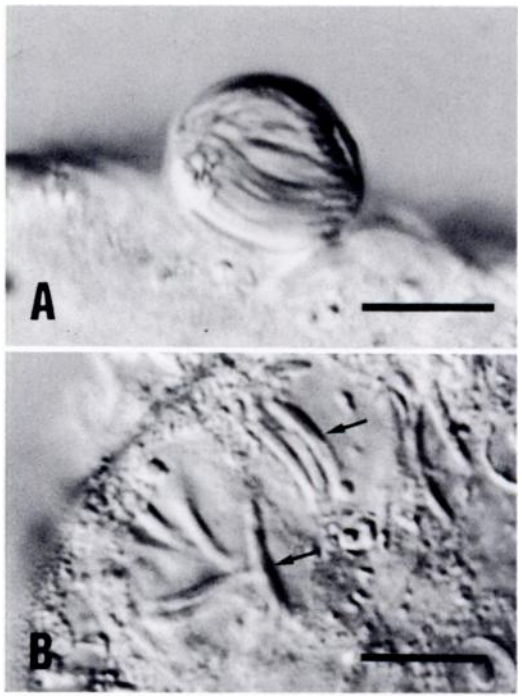


FIGURE 1. Sporogonic stages of *Haemoproteus meleagridis* from *C. edeni*, 7 days after fly engorged on infected turkey. **A.** Mature oocyst. **B.** Sporozoites (arrows) in a salivary gland. Nomarski interference-contrast microscopy. Bars = 10 μm.

sis. Development was not observed in a single specimen of *C. crepuscularis* and two specimens of *C. haematopotus*.

Sporozoites in salivary glands from *C. edeni*, *C. hinmani* and *C. arboricola* infected 12 of 20, one of six and one of two turkey poults, respectively, when inoculated i.p. or i.v. The prepatent period for the 14 successful infections ranged from 17 to 18 days. This figure is less than the 28 day prepatent period that Greiner and Forrester (1980, op. cit.) estimated from observations of sentinel turkeys exposed to vectors. It is similar to the shorter prepatent periods of other avian haemoproteids transmitted by *Culicoides*; 14–21 days for *H. nettionis* Johnston and Cleland (Fallis and Wood, 1957, Can. J. Zool. 35: 425–435), 14 days for *H. mansoni* Sanbon (Fallis and Bennett, 1960, Can. J. Zool. 38: 455–464) and 11–14 days for *H. velans* Coatney and Rondabush (Khan and Fallis, 1971, Can. J. Zool. 49: 420–421).

The technical problems inherent in handling sticky salivary glands that are only 200–300 μm long may account for the fairly low (50%) success rate of the experimental infections. Another possibility is that the sporozoites observed in these wild flies belonged to another species of haemosporidian. However, in almost 500 dissections of *Culicoides* that were captured unengorged or that engorged on uninfected bait turkeys, sporozoites were found in only one fly (*C. edeni*) in spite of the fact that collections were made during periods of active *H. meleagridis* transmission. The identity of the sporozoites was not determined.

These observations add *H. meleagridis* to the group of avian haemoproteids that are transmitted by species of *Culicoides*. Other species of *Culicoides* that have been identified as vectors of *Haemoproteus* include *C. nubeculosus* (Meigen) for *H. desseri* Miltgen, Landau, Ratanaworabham, and Yenbutra (Miltgen et al., 1981, Ann. Parasitol. Hum. Comp. 56: 123–130), *C. crepuscularis* for *H. fringillae* Labbé (Fallis and Bennett, 1961, Can. J. Zool. 39: 215–228) and *H. danilewskyi* Kruse (Bennett and Fallis, 1960, Can. J. Zool. 38: 261–273), *C. downesi* Wirth and Hubert for *H. nettionis* (Fallis and Wood, 1957, op. cit.), *C. stilobezzioides* Foote and Pratt for *H. velans* (Khan and Fallis, 1971, op. cit.) and *H. danilewskyi* (Bennett and Fallis, 1960, op. cit.) and *C. sphagnumensis* Williams for *H. velans* (Khan and Fallis, 1971, op. cit.), *H. mansoni* (Fallis and Bennett, 1960, op.

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cit.) and *H. danilewskyi* (Fallis and Bennett, 1961, op. cit.). Neither *Culicoides edeni*, *C. hinmani* nor *C. arboricola* have been previously implicated as vectors of haemosporidian parasites. Besides *C. crepuscularis*, none of the previously proven vectors are present in Florida.

The Bennett trap collections of *Culicoides* attracted to bait turkeys are the first biting records for *C. edeni* and *C. nanus* and the first biting record of *C. baueri* for birds. Biting collections of the remaining species have been made from both birds and mammals (Blanton and Wirth, 1979, The Sand Flies (*Culicoides*) of Florida, Fl. Dept. Agric. Consumer Services, Gainesville, Florida, 204 pp.).

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Comparative Morphology of the Eggs of the Paramphistomid Trematodes of the Agile Wallaby, *Macropus agilis* (Gould, 1842)

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The paramphistomid trematodes, *Gemellicotyle wallabicola* Prudhoe, 1975 and *Macropotrema pertinax* Blair, Beveridge and Speare, 1979 are both parasites of the agile wallaby. *Gemellicotyle wallabicola* which occurs in the stomach, was first described from the agile wallaby from the Bula Plains in Papua New Guinea (Prudhoe, 1975, Dr. B. S. Chauhan, Comm. Vol., pp. 63–68), while *M. pertinax*, which inhabits the cecum and colon, was described from four locations in northern Australia (Blair et al., 1979, Ann. Parasitol. Hum. Comp. 54: 585–592). In a survey of parasites of agile wallabies (Speare et al., 1983, Aust. Wildl. Res. 10: 89–96), *G. wallabicola* was found near Darwin (Northern Territory) and at Ingham and at Stone's Crossing, Wenlock River (Queensland). In the same survey, *M. pertinax* was found in wallabies from near Darwin (Northern Territory) and from Cardwell, Ingham, Stone's Crossing and Townsville (Queensland). The geographical ranges of these paramphistomes overlap and may with further collecting prove to be the same. As the life cycles of both paramphistomes and the significance of their associated pathological changes are unknown, it may prove useful to be able to identify naturally infected wallabies prior to necropsy. This paper compares the morphology of the eggs of both species and describes their differentiation in fecal samples.

A mature, wild female *M. agilis* was collected after being killed by a motor vehicle 15 km south of Ingham, Queensland. At necropsy, nine *G. wallabicola* were found in the stomach and 120 *M. pertinax* were recovered from the cecum and upper 15 cm of colon. These live parasites were washed in normal saline until free of gut contents and three *G. wallabicola* and 20 *M. pertinax* were selected randomly and placed separately in two petri dishes containing 0.85% saline. The petri dishes were kept at 22 C for 6 hr, the trematodes removed, and the eggs stored in saline at 4 C for a further 6 hr. Twenty-five eggs of each species were chosen at random and measured using an ocular micrometer, measurements being given in μm as mean $\pm$ standard deviation (range).

Direct fecal smears were examined from the original wallaby and from a second wild agile

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