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Hematozoa of Waterfowl from Michigan

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ABSTRACT: Two hundred eighteen and 127 wild waterfowl (Anatidae) of five species were sampled from the Kellogg Biological Station area (Michigan, USA) during the summer (1 June to 24 August 1995) and fall (9 September to 8 October 1995), respectively. Twelve (6%) of those sampled in summer and 13 (11%) sampled in the fall were infected with hemosporids. *Haemoproteus nettionis, Haemoproteus greineri,* and *Leucocytozoon simondi* infected both summer and fall birds, with *H. nettionis* the most common (4% summer; 7% fall). Mean intensities were low; the highest mean intensity was 4.6 ± 1.1 gametocytes per 5,000 uninfected erythrocytes for *H. nettionis* in summer. Of 123 local waterfowl, none were infected with any blood parasite. Thirty-five captive year-round resident waterfowl also were sampled and no blood parasites were found.

Key words: Anatidae, hematozoa, *Haemoproteus nettionis, Haemoproteus greineri, Leucocytozoon simondi,* survey.

Hematozoan parasites of waterfowl are believed to be nearly global in distribution and found wherever suitable waterfowl hosts and insect vectors are found (Greiner et al., 1975). However, there are many locations where waterfowl have infrequently or never been surveyed for blood parasites. In Michigan (USA), surveys of wild waterfowl have been few and limited to the Douglas Lake area in the northern lower peninsula (O'Roke, 1931, 1934; Chernin and Sadun, 1949) and the Seney National Wildlife Refuge in the upper peninsula (Herman et al., 1975). There also have been some transmission-related studies of waterfowl hematozoa at Douglas Lake (O'Roke, 1931, 1934; Chernin, 1956a; Barrow et al., 1968) and at various locations in the upper peninsula (Tarshis, 1976; Desser et al., 1978; Sibley and Werner, 1984). The only report of waterfowl hematozoa in the southern lower peninsula of Michigan is a note (Kocan, 1966) of a migrating canvasback (*Aythya valisneria*) infected with *Plasmodium* sp. from the Kellogg Bird Sanctuary, part of the W. K. Kellogg Biological Station (KBS) of Michigan State University in southwestern Michigan (42°24′N, 85°24′W).

The present study was undertaken to survey the hematozoa of waterfowl in the KBS area. This study focused on three locations within or adjacent to KBS: the Kellogg Bird Sanctuary (which includes Wintergreen Lake, a 15 ha hypereutrophic lake), the Kellogg Farm (which includes several eutrophic ponds), and Gull Lake (a 822 ha oligotrophic lake bordering KBS). The KBS area has large, high-density waterfowl populations during the summer consisting of approximately 50 wood ducks (*Aix sponsa*), 200 mallards (*Anas platyrhynchos*), and 100 Canada geese (*Branta canadensis*) at the Bird Sanctuary alone; during the migratory seasons there are over 3,000 Canada geese, 2,000 wood ducks, mallards, and other duck species, and 50 swan (various species) at the Bird Sanctuary. We hypothesized that waterfowl in the KBS area would have large and interactive hematozoan communities because of the large waterfowl populations and the many aquatic habitats which might support vector populations.

Blood was collected from 218 wild waterfowl captured with drive traps and bait traps and banded in the KBS area from June 1 to August 24, 1995. Additionally, 35 captive waterfowl of native and exotic species (black duck [*Anas rubripes*], Northern pintail [*Anas acuta*] hybrid, black swan [*Cygnus atratus*], domestic mallard, domestic greylag goose [*Anser anser*], mallard, mute swan [*Cygnus olor*], trumpeter swan [*Cygnus buccinator*]), which were year-round residents of the Kellogg Bird Sanctuary, were sampled for blood during

this time. To see if migrant birds had similar or different levels of hematozoa infection, blood was collected from 127 wild waterfowl captured by bait trap and banded at Kellogg Bird Sanctuary from 9 September to 8 October 1995 (dates chosen by observation of large numbers of fall migrants). Only 13 of these were recaptures of birds sampled and banded in the summer; these were not included in comparisons of summer and fall birds. Waterfowl species, sex, and age (after-hatch-year = AHY, hatch-year = HY, and HY birds that had not yet attained flight were local = L) were identified at the time of capture. All L birds in this study were at least four weeks old. Birds were bled at the capture site using a sterile lancet to prick the metatarsal vein. Two thin blood smears were made in the field for each bird, air dried, and fixed and stained using Fisher Scientific LeukoStat™ Stain Kit (Fisher Scientific, Pittsburgh, Pennsylvania, USA).

To determine prevalence of hematozoans, blood smears were first scanned in their entirety at 200×. Then they were examined at 1,000× magnification for 10 min each, so that a total of 20 min was expended to examine both slides from each bird. Prevalence is defined as the number of birds infected with a particular hematozoan species divided by the number of birds examined. Overall hematozoan prevalence is defined as the number of birds infected with any hematozoan species divided by the number of birds examined. Representative specimens from infected birds are deposited at the International Reference Center for Avian Hematozoa (Queensland Museum, University of Queensland, Queensland, Australia; accession numbers G463097, G463098, G463101 for *Haemoproteus nettionis,* G463102 for *H. greineri,* G463099 for *Leucocytozoon simondi,* G463100 for unidentified microfilaria).

If hematozoans were present, the best slide was selected (based on smear thickness and staining) and 5,000 erythrocytes were counted in 50 replicates of 100 erythrocytes each (at 400×) to provide an estimate of parasite intensity within each infected bird (Fedynich et al., 1995). Each count of 100 erythrocytes (one replicate) was obtained using one or more different fields of view delineated by a Miller ocular disc (Klarmann Rulings, Inc., Manchester, New Hampshire, USA). A random number table (Rohlf and Sokal, 1981) was used to determine the number of fields skipped between each field of view examined. If a field of view was inadequate for examination (e.g., too thick) the smear was advanced to the next suitable field of view. Intensity is defined as the number of erythrocytes infected by a hematozoan species divided by 5,000 erythrocytes counted in a particular host. Intensities $<1/5{,}000$ erythrocytes were arbitrarily assigned a value of 0.5 so that they could be included in the analysis (Fedynich and Rhodes, 1995). Chi-square tests were used to compare prevalences and because intensity data were not normally distributed, Kruskal-Wallis or Mann-Whitney *U* tests were used in their analysis. All statistical analyses were performed using SYSTAT For Windows, v.6.0.1 (SPSS, Inc., Chicago, Illinois, USA).

Leucocytozoon spp. have rarely been quantified on blood smears because of concern over potential pooling of this parasite on the smear and because *Leucocytozoon* spp. infect leukocytes in addition to erythrocytes (Fedynich et al., 1995). Fedynich and Rhodes (1995), however, tested for pooling and found *Leucocytozoon smithi* densities to vary concordantly with erythrocyte densities, allowing for reliable quantification of mean intensity. *Leucocytozoon simondi* intensities in this study were too low to test this assumption.

In wild waterfowl sampled in the summer, three hematozoan species, *Haemoproteus nettionis, H. greineri,* and *Leucocytozoon simondi,* were present, with prevalences for all waterfowl species combined 4%, <1%, and 2%, respectively (Table 1). Overall hematozoan prevalence was 6%. One mallard was infected with both

TABLE 1. Number of waterfowl sampled, overall hematozoan prevalence (%), and (number of birds infected) by species, age, and season.

Species	Summer[a]				Fall[b]				Total
	L[c]	HY[d]	AHY[e]	Subtotal	L	HY	AHY	Subtotal	
Black duck	—	—	1 0% (0)	1 0% (0)	—	1 100% (1)	1 0% (0)	2 50% (1)	3 33% (1)
Canada goose	49 0% (0)	—	28 11% (3)	77 4% (3)	—	—	—	—	77 4% (3)
Mallard	73 0% (0)	14 14% (2)	32 19% (6)	119 7% (8)	—	77 9% (7)	24 13% (3)	101 10% (10)	220 8% (18)
Mallard-black duck hybrid	—	—	—	—	—	—	2 50% (1)	2 50% (1)	2 50% (1)
Wood duck	1 0% (0)	10 0% (0)	10 10% (1)	21 5% (1)	—	3 0% (0)	6 17% (1)	9 11% (1)	30 7% (2)
Total	123 0% (0)	24 8% (2)	71 14% (10)	218 6% (12)	—	81 10% (8)	33 15% (5)	114 11% (13)	332 8% (23)

[a] 1 June to 24 August 1995.
[b] 9 September to 8 October 1995.
[c] Local.
[d] Hatch-year.
[e] After-hatch-year.

H. nettionis and *L. simondi,* and one wood duck was infected with both *H. nettionis* and *H. greineri.* Canada geese were infected only with *H. nettionis.* The 35 captive waterfowl were not infected with any hematozoan.

The same three hematozoan species, *H. nettionis, H. greineri,* and *L. simondi,* were found in fall waterfowl, with prevalences for all waterfowl species combined 7%, 4%, and 5%, respectively (Table 1). Fall waterfowl had an overall hematozoan prevalence of 11%, almost 2× that of summer waterfowl, but this difference was not significant (Table 2; $\chi^2 = 2.9$, $P = 0.09$). Four mallards had dual infections; two were infected with *H. nettionis* and *H. greineri,* and two were infected with *H. nettionis* and *L. simondi.* One mallard was infected with three parasites, *H. nettionis, L. simondi,* and an unidentified microfilaria. No infections were detected in the 13 birds banded in the summer and recaptured in the fall.

Low prevalences of individual hematozoan species precluded statistical analysis of the effects of host-intrinsic factors on prevalence within each host species. However, using overall hematozoan prevalence, comparisons between waterfowl species were made and no differences were found in either summer or fall ($\chi^2 = 0.8, 6.12$, $P > 0.05$). Therefore, data from different waterfowl species were combined and comparisons between waterfowl age and sex were made. In the summer, no L waterfowl were infected with any blood parasite. In both summer and fall overall hematozoan prevalence did not differ significantly between HY and AHY classes ($\chi^2 = 2.1, 0.3$, $P > 0.05$) or between sexes ($\chi^2 = 0.4, 0.6$, $P > 0.05$).

In both seasons, mean intensities of hematozoans were low (Table 1). The low prevalences of hematozoans precluded statistical analyses of the effects of host-intrinsic factors on intensities within and between host species. When data from waterfowl species were combined, host-intrinsic factors were analyzed using intensities, but no significant differences were found for mean intensities of any parasite between waterfowl ages ($U = 1.0, 0.5$, $P > 0.05$) or sexes ($U = 1.0, 7.0$, $P > 0.05$). Mean intensity of *H. nettionis* was significantly higher in the summer than in the fall ($U = 47.0$, $P = 0.02$), but no such differences existed for *H. greineri* or *L. simondi* ($U = 0.0, 15.0$, $P > 0.05$).

The present study is the first survey of waterfowl from the KBS area and from southern Michigan and is the first report of hematozoa in waterfowl breeding in southern Michigan. The three hematozoan species found in this study have been previously reported from northern Michigan waterfowl (O'Roke, 1931, 1934; Chernin and Sadun, 1949; Herman et al., 1975; Desser et al., 1978; Sibley and Werner, 1984), but comparatively, prevalences of hematozoans in the KBS areas were low. Herman et al. (1975) reported *L. simondi* as the causative agent of Canada geese gosling mortality at the Seney National Wildlife Refuge in the upper peninsula. They reported prevalence of 80% in AHY geese just prior to egg-laying and 100% in goslings each year. They also found *H. nettionis, Plasmodium* spp., and trypanosomes, but stated that prevalences were low compared to *L. simondi.* O'Roke (1931, 1934) found that 100% of black ducks and mallards were infected in the Douglas Lake vicinity and 30% of black ducks were infected in the Munuscong Lake vicinity. It appears that southern Michigan waterfowl populations may have lower blood parasite loads than waterfowl populations in northern Michigan.

Mean intensities of hemosporids also were low. The highest mean intensity value was for *H. nettionis* in the summer, and this was significantly higher than in the fall. In temperate regions hemosporid intensities are typically highest during the breeding season, enhancing the probability of transmission at the time new hosts are available (Chernin, 1956b; Atkinson and van Riper, 1991). *Haemoproteus nettionis*

TABLE 2. Prevalence, (number of birds infected), mean intensity ± SD, and (intensity range) of hematozoan species in wild waterfowl during summer and fall, 1995. See text for genus abbreviations.

	Summer[a]			Fall[b]		
Waterfowl species	*H. nettionis*	*H. greineri*	*L. simondi*	*H. nettionis*	*H. greineri*	*L. simondi*
Black duck	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	50% (1)
Canada goose	4% (3) 4.7 ± 2.2 (2–9)	0% (0)	0% (0)	—	—	—
Mallard	4% (5) 5.2 ± 1.6 (1–10)	0% (0)	3% (4) 0.9±0.1 (0.5–1)	8% (8) 1.3±0.2 (0.5–2)	2% (2) 4.0±0 (4)	5% (5) 0.8±0.1 (0.5–1)
Mallard-black duck hybrid	—	—	—	0% (0)	50% (1) 1.0 (1)	0% (0)
Wood duck	5% (1) 1.0 (1)	5% (1) 0.5 (0.5)	0% (0)	0% (0)	11% (1) 1.0 (1)	0% (0)
Total	4% (9) 4.6 ± 1.1 (1–10)	<1% (1) 0.5 (0.5)	2% (4) 0.9 ± 0.1 (0.5–1)	7% (8) 1.3 ± 0.2 (0.5–2)	4% (4) 2.0 ± 0.7 (1–4)	5% (6) 0.8 ± 0.1 (0.5–1)

[a] 1 June to 24 August 1995.
[b] 9 September to 8 October 1995.

was the only parasite species to exhibit this pattern, however.

Haemoproteus spp. are common and widespread in North American waterfowl (Greiner et al., 1975) and use *Culicoides* spp. as vectors (Meyer and Bennett, 1976). Sibley and Werner (1984) reported natural transmission of *H. nettionis* by *Culicoides downesi* to domestic ducks in the upper peninsula of Michigan. It is notable that they observed gametocytes which completely encircled the host cell nucleus in addition to halteridial gametocytes, but identified all of them as *H. nettionis,* because *H. greineri* had not yet been described (described in Bennett et al., 1984). We suggest that *H. greineri,* which has gametocytes that completely encircle the host nucleus, was also transmitted in their study and that the Michigan upper peninsula may be part of the transmission range of this parasite. Although Bennett et al. (1984) suggested that *H. greineri* was endemic to the prairie regions of Canada, a recent study extended the known transmission range of *H. greineri* to include eastern Canada and northeastern United States (Pung et al., 1997). In the present study, some of the birds infected with *H. greineri* were HY, but none were L, so it is not clear if southern Michigan is also part of the transmission range of this parasite.

Low prevalences of *L. simondi* were not unexpected because swift, highly oxygenated stream habitat required by the larvae of black flies, who as adults are vectors of *L. simondi,* is not common in southwestern Michigan. Black fly larvae were observed on a water level control gate on Wintergreen Lake in June 1995. Because of their known affinity for lake outlets, these larvae were likely *Simulium decorum,* a species not known to vector avian blood parasites (Crosskey, 1990).

Plasmodium spp. were not detected in any waterfowl. The lack of *Plasmodium* spp. infections, the low overall hematozoan prevalence, and the low mean intensities were surprising given the large, high-density waterfowl populations and the abundance and variety of aquatic habitats suitable for vector larvae (with the exception of swift streams for black flies) in the KBS area.

The lack of any infections in 123 L waterfowl sampled suggests that transmission of hematozoa in the KBS area may have been infrequent or absent in 1995. The low prevalences in older birds and the lack of infection in 35 captive birds also suggest that transmission has been infrequent in previous years. We were unfortunately unable to conduct surveys in additional years to determine whether 1995 was an abnormal year in regards to hematozoan transmission and prevalence. Meteorological data for 1995 show that monthly temperatures and precipitation were within 16% of average in March to August (http://lter.kbs.msu.edu). These data suggest that vectors, if present, should not have been drastically affected by weather in 1995.

The hematozoan community of KBS waterfowl in 1995 can be characterized as isolationist, having low prevalences and intensities, a low number of multiple infections, and probably little interaction between hematozoan species. Similarly, Fedynich et al. (1993) classified the hemosporid community of waterfowl wintering in south Texas as isolationist based on low prevalences, low intensities, and low number of multiple infections.

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