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## Hematozoa of Hatch-year Common Mergansers from Michigan

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**ABSTRACT:** Fifty-five hatch-year common mergansers (*Mergus merganser*) were sampled for hematozoa from Douglas Lake (Michigan, USA) on 17 July 1995. Forty-one (75%) were infected with hematozoa. *Haemoproteus greineri* and *Leucocytozoon simondi* were common, infecting 28 (51%) and 26 (47%) common mergansers, respectively. *Plasmodium circumflexum* infected two (4%) birds. The common merganser is a new host record for *H. greineri* and *P. circumflexum*. Intensity data indicate possible negative interspecific interaction between *H. greineri* and *L. simondi*.

**Key words:** waterfowl, Anatidae, survey, *Haemoproteus greineri*, *Leucocytozoon simondi*, *Plasmodium circumflexum*, interspecific interaction.

Douglas Lake, located in the northern lower peninsula of Michigan (USA; 45°36'N, 84°42'W) and the University of Michigan Biological Station (UMBS) which is located on the lake's southern shore, have had an important historical role in the investigation of avian hematozoa, particularly the species *Leucocytozoon simondi* which infects waterfowl (Anatidae). O'Roke (1934), working at UMBS, described *L. simondi* (using the synonym *L. anatis*) in detail. In addition to this description, O'Roke, (1931, 1934) elucidated a great deal of information about *L. simondi*, including all life cycle stages, the time required for the development of different life stages, identification of *Simulium* spp. as vectors, vector feeding ecology, and the pathology, morbidity, and mortality caused by the parasite in ducks. Other important studies completed at UMBS include Chernin (1956b), which showed that the seasonal increase in parasitemia, or spring relapse, in *L. simondi* coincides with egg-laying in ducks, and Barrow et al. (1968), which focused on the feeding habits of the black fly *Simulium rugglesi* and

the transmission of *L. simondi* in the Douglas Lake area by this vector.

O'Roke (1931, 1934) found high prevalences of *L. simondi* (up to 100%) in wild black ducks (*Anas rubripes*) and mallards (*Anas platyrhynchos*) from Douglas Lake and stated that brood sizes were noticeably reduced by *L. simondi*-caused mortality. O'Roke (1934) and Chernin (1956a) found prevalences near 100% in farm-raised Pekin ducks (*Anas platyrhynchos*) from the UMBS area. One of us has observed *L. simondi* in hatch-year (HY) mallards from Douglas Lake (H. D. Blankespoor, unpubl. data) and possible associated mortality of HY mallards over the last two decades. Our casual observation that HY common mergansers on Douglas Lake do not appear to experience such mortality, caused us to wonder whether they were infected. Common mergansers are difficult to capture; therefore, they have rarely been surveyed for hematozoa (Greiner et al., 1975). The goal of the present study was to survey the common mergansers on Douglas Lake for hematozoa to add to the known information about common merganser parasites and to the history of waterfowl hematozoa in the Douglas Lake area.

Blood was collected from 55 HY common mergansers captured using a drive trap and banded on 17 July 1995. All birds were members of a single creche formed from four natural broods. They were approximately 6-wk-old and unable to fly. Sex was determined at the time of capture. Birds were bled using a sterile lancet to prick the metatarsal vein. Two thin blood smears were made for each bird, air dried, fixed, and stained using Fisher Scientific LeukoStat<sup>TM</sup> Stain Kit (Fisher Scientific, Pittsburgh, Pennsylvania, USA).

To determine prevalence of hematozoa,

blood smears were first scanned in their entirety at 200 $\times$ . Then they were examined at 1,000 $\times$  magnification for 10 min each, so approximately 250–350 fields of view, or 15,000–25,000 erythrocytes were viewed. 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.

If hematozoa 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 $\times$ ) 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 host cells infected by a hematozoan species divided by 5,000 erythrocytes counted in a particular host. Intensities <1/5,000 erythrocytes were assigned a value of 0.5 so that they could be included in the analysis (Fedynich and Rhodes, 1995). Contingency analyses were used to analyze prevalences and the effects of host sex. Because intensity data were not normally distributed, 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).

Representative specimens from infected birds are deposited at the International Reference Center for Avian Hematozoa (Queensland Museum, University of Queensland, Queensland, Australia; acces-

sion numbers, G463094 and G463096 for *Haemoproteus greineri*, G463093 for *Leucocytozoon simondi*, G463096 for *Plasmodium circumflexum*).

*Leucocytozoon* spp. have rarely been quantified on blood smears because of concern over potential non-random distribution 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 non-random distribution and found *Leucocytozoon* densities varied concordantly with erythrocyte densities, allowing for reliable quantification of mean intensity. Based on regression analysis from a subsample of ten smears from ten common mergansers from Douglas Lake, *L. simondi* densities varied concordantly with erythrocyte densities (data not shown). Thus, the number of *L. simondi* gametocytes per 5,000 uninfected erythrocytes appears to be an accurate measure of intensity.

Three hematozoan species, *Haemoproteus greineri*, *L. simondi*, and *Plasmodium circumflexum* were identified. *Haemoproteus greineri* and *L. simondi* were the most common, detected in 28 (51%) and 26 individuals (47%), respectively. Thirteen individuals (24%) were infected with *H. greineri* and *L. simondi*. *Plasmodium circumflexum* was detected in only two individuals (4%) and occurred only as dual infections with *H. greineri*. Overall hematozoan prevalence was 75% (41 individuals). Prevalences did not differ between host sexes for overall or specific hematozoan prevalence ( $\chi^2 = 0.005$ – $2.2$ ,  $P > 0.05$ ). The number of dual infections of *H. greineri* and *L. simondi* (13) was not different than that expected by chance (13; obtained by multiplying the prevalences of the two species). *Plasmodium circumflexum* prevalences were not analyzed because only two birds were infected.

Mean intensities of either *H. greineri* ( $2.8 \pm 3.3$ ; mean  $\pm$  SD) or *L. simondi* ( $4.5 \pm 15.1$ ) did not differ significantly between host sexes ( $U = 74.5$ – $95.0$ ,  $P >$

0.05). *Plasmodium circumflexum* mean intensity ( $1.3 \pm 1.1$ ) was excluded from statistical analysis because of the low number of birds infected. Mean intensities of *H. greineri* and *L. simondi* were not significantly different ( $U = 443.5$ ,  $P > 0.05$ ). However, *H. greineri* mean intensity in single infections (*H. greineri* only;  $4.0 \pm 4.1$ ) was significantly greater than *H. greineri* mean intensity in dual infections with *L. simondi* ( $1.5 \pm 1.5$ ;  $U = 123.5$ ,  $P = 0.04$ ). *Leucocytozoon simondi* mean intensities did not differ between single ( $7.4 \pm 21.3$ ) and dual infections with *H. greineri* ( $1.6 \pm 1.7$ ;  $U = 80.0$ ,  $P > 0.05$ ).

This study more than doubles the number of common mergansers surveyed for hematozoa and is the first such study to include intensity data. Greiner et al. (1975), summarizing data known for all North American avian hematozoa, report overall hematozoan prevalence in common mergansers as 41% (14 infected of 34 sampled), with prevalences of *Haemoproteus* spp. and *L. simondi* 35% and 9%, respectively.

The high prevalence of *L. simondi* in hatch-year common mergansers from Douglas Lake is consistent with the high prevalences previously found in black ducks and mallards from Douglas Lake (O'Roke, 1931, 1934) and in waterfowl from other areas in the northern lower and upper peninsulas of Michigan (O'Roke, 1931, 1934; Chernin, 1956b; Herman et al., 1975). The results presented here show that *L. simondi* is still prevalent in the Douglas Lake area more than sixty years after O'Roke's work (1931, 1934) and that *Simulium* spp. vectors are present to transmit the parasite. The high prevalences of *H. greineri* and *L. simondi* and the high overall hematozoan prevalence (75%) in Douglas Lake common mergansers are in contrast to low prevalences in other species of waterfowl in southwestern Michigan (DeJong and Muzzall, 2000).

Our previous observation that common mergansers on Douglas Lake do not seem to experience the mortality caused by *L.*

*simondi* seen in mallards remains unexplained. Species differences in host immune defenses may explain this observation. The almost exclusive fish diet of mergansers is higher in protein and iron than the invertebrate and primarily vegetation diet of mallards (Bellrose, 1980). Protein has been shown to be important in the immunocompetence of birds (Lochmiller et al., 1993). Gonzalez et al. (1999) found that house sparrows fed a high-protein diet had a higher cellular immune response and a higher cellular immune response conferred a higher probability of recovering from infection with *Haemoproteus* sp. Iron as well as protein may be especially important in combating anemia, which is a cause of death in mallards infected with *L. simondi* (Kocan and Clark, 1966). In Michigan, another difference between common mergansers and mallards is that the former are much more commonly and heavily infected with avian schistosomes (Blankespoor and Reimink, 1991). It is possible infection with schistosomes, which like hematozoa live in the blood stream, affects the pathogenicity of *L. simondi*, either directly, or indirectly through stimulation of the host immune system.

The common merganser is a new host record for *H. greineri*. *Haemoproteus nettionis* has been reported in waterfowl from the northern lower peninsula (Chernin and Sadun, 1949) and from the upper peninsula of Michigan (Herman et al., 1975; Sibley and Werner, 1984). *Haemoproteus greineri* was formerly thought to be endemic to the prairie regions of Canada (Bennett et al., 1984), but a recent study (Pung et al., 1997) extended the known range of this parasite to include the eastern provinces of Canada and the northeastern United States. The presence of *H. greineri* in HY common mergansers indicates transmission of this parasite at Douglas Lake and is consistent with the suggestion of DeJong and Muzzall (2000) that Sibley and Werner (1984) observed transmission of *H. greineri* to domestic ducks

in addition to *H. nettionis* in Michigan's upper peninsula (*H. greineri* had not been described at the time of Sibley and Werner's work). *Culicoides* spp., the vectors of *Haemoproteus* spp. of waterfowl (Fallis and Wood, 1957) have been reported feeding from waterfowl at Douglas Lake (Barrow et al., 1968) and *C. downesi* has been reported as the vector of *Haemoproteus* spp. in the upper peninsula (Sibley and Werner, 1984).

The common merganser is a new host record for *P. circumflexum*; furthermore, this is the first record of any *Plasmodium* spp. in common merganser. Although prevalence was low, the presence of *P. circumflexum* in HY common mergansers indicates the presence of the correct insect vectors (presumably *Culex* spp. or *Culiseta* spp.; Meyer and Bennett, 1976) at Douglas Lake.

Very few investigators have examined prevalence data for interspecific associations among hematozoan parasites (Forbes et al., 1994) and none have examined mean intensity data, although some have noted the potential for interspecific interaction at the component community level (e.g., Fedynich et al., 1995). Forbes et al. (1994), studied blood parasites of a blue grouse (*Dendragapus obscurus*) population and found negative associations (fewer joint presences than expected by chance) of *Haemoproteus* sp. with microfilariae and *Haemoproteus* sp. with *Leucocytozoon* sp. We found no such negative associations in our prevalence data.

Interestingly however, mean intensity of *H. greineri* in dual infections with *L. simondi* was significantly less than mean intensity of *H. greineri* in single infections. This result shows a negative interaction between hematozoan species, with *L. simondi* having a detrimental effect on *H. greineri*. This effect could be due to direct competition for host cells at either or both of the schizont and gametocyte stages. Another possibility is that the effect is immuno-mediated, with *L. simondi* evoking a host immune response that is detrimen-

tal to *H. greineri*. In this case, it may be important which parasite invades the host first, which may depend upon vector emergence periods. Although this effect is statistically significant, it is based on a small sample size, and future studies of waterfowl hematozoa are needed to confirm or refute the existence of an interspecific interaction between *H. greineri* and *L. simondi*. Our detection of a possible interaction in intensity data but not in prevalence data highlights the importance of measuring intensities in such studies.

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