Hematologic Parameters and Hemoparasites of Nonmigratory Canada Geese (*Branta canadensis*) From Greensboro, North Carolina, USA

Author(s): Lauren E. Charles-Smith , MS, DVM, M. Elizabeth Rutledge , MS, PhD, Caroline J. Meek , Katherine Baine , DVM, Elizabeth Massey , DVM, Laura N. Ellsaesser , DVM, Christopher S. DePerno , MS, PhD, Christopher E. Moorman , MS, PhD, and Laurel A. Degernes , DVM, MPH, Dipl ABVP (Avian)

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Lauren E. Charles-Smith, MS, DVM, M. Elizabeth Rutledge, MS, PhD, Caroline J. Meek, Katherine Baine, DVM, Elizabeth Massey, DVM, Laura N. Ellsaesser, DVM, Christopher S. DePerno, MS, PhD, Christopher E. Moorman, MS, PhD, and Laurel A. Degernes, DVM, MPH, Dipl ABVP (Avian)

Abstract: Large flocks of wild, nonmigratory Canada geese (*Branta canadensis*) have established permanent residence throughout the eastern United States and have become a public concern. Few studies have assessed the hematologic parameters for these populations, which could provide useful information for monitoring individual and population health of Canada geese. This study measured the hematologic parameters and detected the presence of hemoparasites from 146 wild, nonmigratory Canada geese in central North Carolina, USA, during their annual molt. The age class, sex, and weight of each bird were recorded at capture. Values for packed cell volume (PCV), estimated white blood cell count, white blood cell differentials, and heterophil : lymphocyte ratios were calculated for each bird. Adults and female geese had higher estimated white blood cell counts compared with juveniles and males, respectively. The PCV increased with weight and age class. Adult geese had higher percentages of heterophils and heterophil : lymphocyte ratios, whereas juvenile geese had higher percentages of lymphocytes. Relative eosinophil counts in adults increased with decreasing bird weight, and relative monocyte counts in juveniles increased with increasing weight. Three percent of geese were infected with species of *Hemoproteus* blood parasites. Atypical lymphocyte morphology, including pseudopods, split nuclei, and cytoplasmic granules, was observed in 5% of the birds. The hematologic values reported for adult and juvenile nonmigratory Canada geese in this study may serve as reference intervals for ecological studies and veterinary care of wild and captive Canada geese.

Key words: hemoparasites, *Hemoproteus,* hematology, avian, Canada goose, *Branta canadensis*

Introduction

Demographic factors, including species, age class, sex, season, and reproductive status, are important when establishing normal hematologic reference intervals for waterfowl.[1–4] These same factors are also widely used to evaluate the health status of individuals or flocks of birds, whether for ecological surveillance or clinical care of captive or wild birds.[5,6] However, no studies exist that have assessed hematologic parameters for populations of wild Canada geese (*Branta canadensis*). The few studies that have been published were based on small numbers of captive geese and may not be representative of wild Canada goose populations.[1,2,7,8] Large flocks of wild, nonmigratory (hereafter, resident) Canada geese have established permanent residence throughout the eastern United States and are of public concern because of their nuisance behaviors and abundance.[9] Because urban and suburban resident geese often live in close proximity to humans and can be infected

From the Department of Clinical Sciences, 1060 William Moore Dr, College of Veterinary Medicine (Charles-Smith, Meek, Baine, Massey, Ellsaesser, Degernes) and Department of Forestry and Environmental Resources, 110 Brooks Ave, College of Natural Resources (Rutledge, DePerno, Moorman), North Carolina State University, Raleigh, NC 27695, USA. Present address: Department of Small Animal Clinical Sciences, 2407 River Dr, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996, USA (Baine); The Exotic Bird Hospital, 8820 Old Kings Rd S, Jacksonville, FL 32257, USA (Massey).

with zoonotic diseases, monitoring the health of goose populations is important for public health. Previous studies have screened resident Canada geese for salmonellosis and campylobacteriosis.[10]

Having a hematologic reference interval for resident Canada geese is useful when monitoring individual and population health parameters. The goal of this study was to develop a hematologic reference database and investigate the presence of hemoparasites in a resident population of adult and juvenile Canada geese during molt.

Materials and Methods

Capture techniques

Over a 3-day period in June 2008, 763 Canada geese were captured during molt (eg, flightless period) at 15 sites in and around Greensboro, NC, USA. The sites were selected based on the presence of geese and included airport property, parks, lakes, golf courses, residential areas, corporate landscapes, and a rock quarry. The geese were corralled from water and/or nearby green space by using walk-in, panel-type traps and were individually marked with neck and leg bands. Capture, handling, and sampling procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Data collection

The geese were vent-sexed (by eversion of the cloaca), aged by plumage (juvenile = hatch year; adult = year after hatch), and weighed. A physical examination, including keel score, evidence of trauma and foot lesions, and an oral examination were conducted. Juvenile geese weighing <1.6 kg were not included in the study.

Blood was drawn from the medial metatarsal vein in approximately 10 apparently healthy birds from each of the 15 sites.[11] A blood volume of 0.1–0.2 mL was collected by using a 1-mL preheparinized syringe with a 25-gauge needle. Immediately after blood collection, 2 hematocrit tubes and 2 blood smears were prepared for each bird.

Blood analysis

Centrifugation was used to determine packed cell volume (PCV) values from hematocrit tubes that were processed and recorded at the end of each collection day. Blood smears were stained (Harleco Wright-Giemsa Stain, Fuccillo Modification EMD No. 64571; EMD Chemicals Inc, Philadelphia, PA, USA) within 1 week of sampling and evaluated microscopically by 1 individual in order to maintain consistency among samples.

In order to estimate the white blood cell (WBC) counts, the monolayer of the smear was examined by using the ×40 objective lens in a stair-step pattern. Only undamaged WBCs in full view were counted from 12 fields, and the high and low counts were discarded. The 10 remaining fields were averaged and multiplied by 2000 in order to determine the total estimated WBC count.[12] All WBC types were reported as a percentage of 100 leukocytes, which were identified in the monolayer by using high-viscosity immersion oil under a ×100 objective lens. Atypical WBC morphology was noted, and blood smears were scanned for the presence of hemoparasites. The incidence of parasite infection was calculated by counting the number of parasites per 2000 red blood cells (RBCs) by using immersion oil under a ×100 objective lens.[13]

Data analysis

Descriptive analyses (eg, mean, standard deviation, minimum, and maximum) were calculated for body weight, PCV, estimated WBC count, WBC differentials, and heterophil : lymphocyte (H : L) ratios. All statistical analyses were conducted by using R64 version 2.14.0.[14] Analysis of variance was used to determine whether age class, sex, or weight was a predictor of estimated WBC count, PCV, or WBC type. Statistical significance was set at $P < .05$.

Results

Blood samples were collected and analyzed from 146 wild, nonmigratory Canada geese captured at 15 locations in Greensboro, NC, during the annual molt. Eighty-five percent (124/146) of the geese sampled were adults, and 14% (21/146) were juveniles (age class data were not recorded for 1 goose). Male (n = 72) and female (n = 73) birds were equally represented (sex was not recorded for 1 goose).

The mean ± SD value of the estimated total WBC for all birds was $18.4 \pm 6.7 \times 10^3$ cells/μL (Table 1). The WBC estimates of 140 birds fell within the 95% confidence interval ($P = .025$). A total of 4% (n = 6) of birds had elevated WBC counts ($32.4–52.6 \times 10^3$ cells/μL), and none of the birds had WBC counts that fell below the confidence interval. Adults had greater estimated WBC counts ($P = .015$), PCV ($P < .001$), heterophil percentage ($P = .002$), and H : L ratio ($P = .003$), and lower lymphocyte percentages ($P <$

Table 1. Mean ± standard deviation and range values for body weight; packed cell volume; estimated total white blood cell count; percentages of heterophils, lymphocytes, eosinophils, monocytes, and basophils; and heterophil : lymphocyte ratio from 146 nonmigratory, wild adult and juvenile Canada geese sampled in Greensboro, NC, USA, during molt.

Parameter	Total (adults and juveniles; n = 146) (mean ± SD)	Adults (n = 124)[a] (mean ± SD)	Range for adults	Juveniles (n = 21)[a] (mean ± SD)	Range for juveniles
Wt (kg)	3.7 ± 0.8	3.9 ± 0.7	2.5–5.4	2.6 ± 0.6	1.6–4.1
PCV (%)	40.3 ± 4.1	40.9 ± 3.7	31–50	37.0 ± 4.3	30–45
Estimated total WBC count ($\times 10^3/\mu L$)	18.4 ± 6.7	18.8 ± 6.9	7.8–52.6	15.7 ± 4.8	5.8–24.6
Heterophil (%)	16.2 ± 9.1	17.1 ± 9.1	1–40	10.5 ± 7.2	0–24
Lymphocyte (%)	60.0 ± 13.7	58.4 ± 13.0	23–87	69.8 ± 13.9	42–95
Eosinophil (%)	20.8 ± 9.7	21.2 ± 9.5	3–62	17.6 ± 10.9	1–37
Monocyte (%)	1.5 ± 2.9	1.6 ± 3.0	0–21	1.1 ± 2.7	0–9
Basophil (%)	1.5 ± 1.5	1.6 ± 1.5	0–8	1.0 ± 1.1	0–3
H : L ratio	0.32 ± 0.3	0.34 ± 0.3	0.01–1.29	0.17 ± 0.1	0–0.45

Abbreviations: SD indicates standard deviation; Wt, body weight; PCV, packed cell volume; WBC, white blood cell; H : L, heterophil : lymphocyte ratio.

[a] The age class for one goose was not recorded.

.001) compared to juveniles (Table 1). The WBC counts in females were greater than those in males (mean: male = 17.1×10^3 cells/μL, female = 19.6×10^3 cells/μL; $P = .010$). Values for PCVs increased with the body weight of adult birds ($P = .004$), whereas eosinophil percentages increased with decreasing body weight in adult birds ($P = .032$). The percentage of monocytes increased with body weight in juvenile geese ($P = .035$). Lymphocyte (Figs 1 and 2), heterophil (Figs 3 and 4), eosinophil (Fig 4), monocyte (Fig 4), thrombocyte (Figs 1, 3, and 5), and basophil (Fig 6) cells were identified in the geese. Atypical lymphocyte cell morphology was observed in 5% (n = 8) of adult geese; these morphologies included split nuclei (Fig 1), pseudopods (Fig 2), and/or purple cytoplasmic granules (Figs 3 and 5).

Hemoproteus species was detected in 3% (n = 4) of the geese, all adults (1 female and 3 males) (Figs 4 and 5). No other blood parasites were observed. The densities of *Hemoproteus* infections were 3, 4, 12, and 59 gametocytes per 2000 RBCs, respectively. The goose (female) with the highest parasite burden had a higher estimated WBC count (42 600 cells/μL) and lower PCV (34%) than male geese that tested positive for *Hemoproteus*, which had an average WBC count of 13 467 cells/μL and an

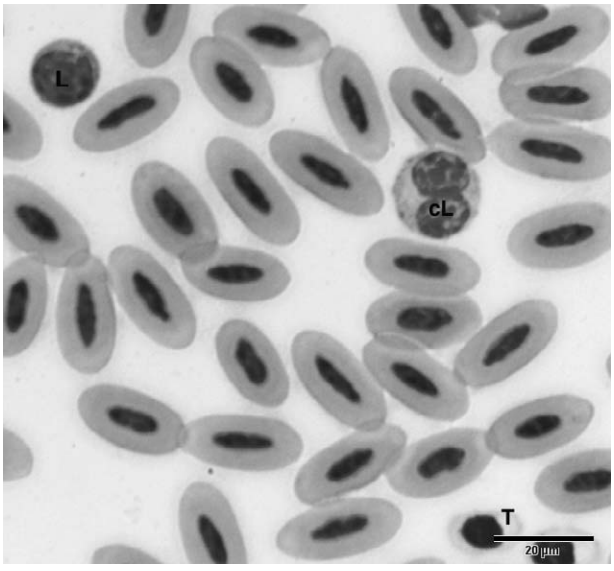

Figure 1. A blood smear from a Canada goose, which includes a lymphocyte (L), a cleaved lymphocyte (cL), and a thrombocyte (T) (Wright-Giemsa; ×100, oil immersion).

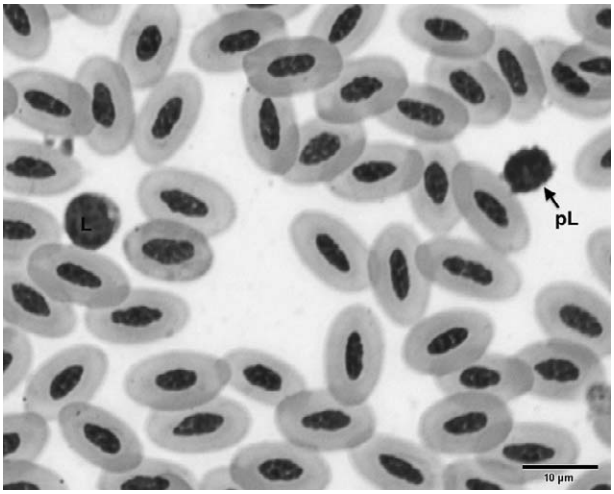

Figure 2. A blood smear from a Canada goose, which includes a lymphocyte (L) and a lymphocyte with pseudopods (pL) (Wright-Giemsa; ×100, oil immersion).

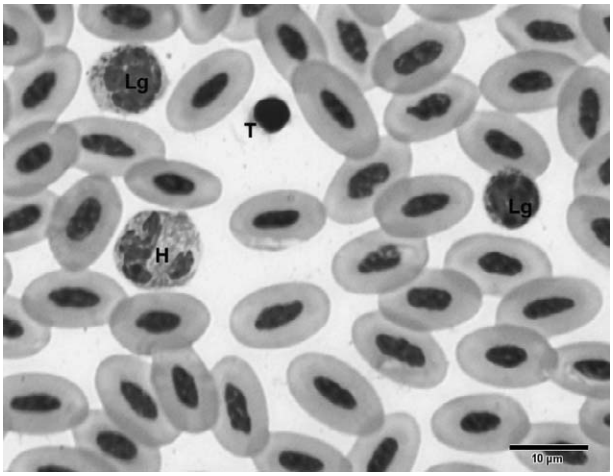

Figure 3. A blood smear from a Canada goose, which includes 2 lymphocytes containing cytoplasmic granules (Lg), a heterophil (H), and a thrombocyte (T) (Wright-Giemsa; ×100, oil immersion).

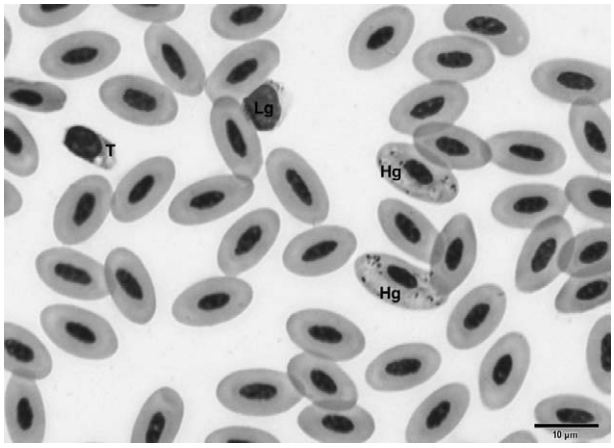

Figure 5. A blood smear from a Canada goose, which includes a lymphocyte containing granules (Lg), 2 erythrocytes infected with *Hemoproteus* gametocytes (Hg), and a thrombocyte (T) (Wright-Giemsa; ×100, oil immersion).

average PCV of 42%. The differences detected in the WBC differential between the female that tested positive for *Hemoproteus* and the mean values from 3 male geese were the percentages of lymphocytes (40% versus 55%, respectively) and eosinophils (33% versus 19%, respectively).

Discussion

To establish normal baseline blood parameters for a species, a large sample size that represents a wide demographic range of animals is needed. Large flocks of wild Canada geese have established permanent residence in central North Carolina and have become a species of public concern because of their nuisance behaviors and abundance.[9] Having the opportunity to collect blood samples from 146 wild Canada geese of different age classes, sex, and from different locations provided an excellent opportunity to establish reference intervals for blood parameters of nonmigratory Canada geese, which can be used in ecological studies and for veterinary care of wild and captive geese.

PCV is an important indicator of hematological status in birds. Our results are consistent with other waterfowl studies that reported higher PCV values in adult birds versus juveniles because of

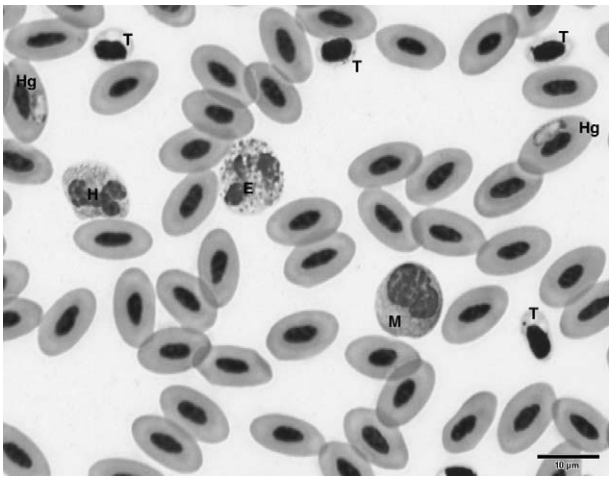

Figure 4. A blood smear from a Canada goose, which includes an eosinophil (E), a monocyte (M), a heterophil (H), 2 immature erythrocytes infected with *Hemoproteus* (Hg), and 4 thrombocytes (T) (Wright-Giemsa; ×100, oil immersion).

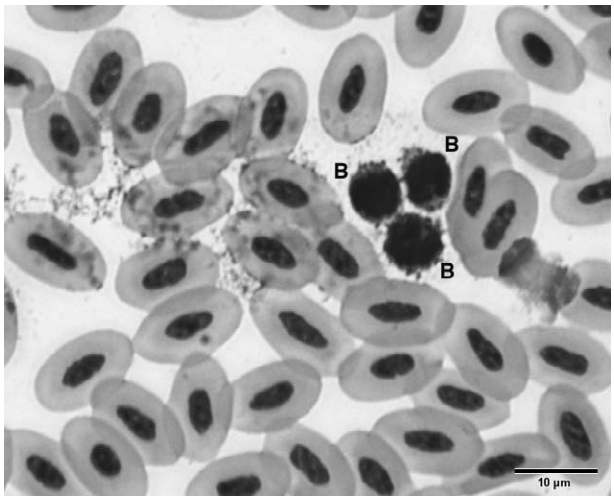

Figure 6. A blood smear from a Canada goose, which includes 3 basophils (B) (Wright-Giemsa; ×100, oil immersion).

increased erythropoesis.[15,16] We observed that PCV values in Canada geese increased with body weight among adults and were similar between males and females during their molt, consistent with other studies.[1,2,8,16] Studies that examined PCV values of Canada geese throughout the year detected increased values during winter and spring months.[1,2] Similar to the case in other avian species, differences in PCV values in Canada geese were most likely a result of molt, reproductive status, ambient temperature, health status, and photoperiod.[17] Therefore, to properly evaluate the PCV values of Canada geese, it is important to consider the time of year as well as the age and weight of individual birds.

Female Canada geese had higher estimated WBC counts compared to male geese in this study. A previous study detected similar hematologic variables between male and female Canada geese, snow geese (*Chen caerulescens*), and blue geese (*Chen caerulescens*) (n = approximately 10 of each sex of each species); however, the year-round average WBC count for female geese was higher than for male geese.[1] WBC counts have been documented to be quite variable between male and females of different indigenous chicken breeds.[18] Although the physiological variables that cause baseline differences between males and females are currently unknown, sex should be taken into account when evaluating the health status of geese based on total WBC counts.

Adult geese had higher estimated WBC counts compared to juvenile geese. Published reference intervals for juvenile Canada geese were not available for comparison, but a significant increase in WBC count with increasing age was reported in Masai ostriches (*Struthio camelus massaicus*).[19] The difference in WBC counts between adult and juvenile geese must be considered when evaluating the health status of a juvenile in order to prevent a misdiagnosis of leukopenia and to recognize true leukocytosis.

A small portion of geese had WBC counts that fell above or below general reference intervals reported for avian species.[1,2,11,20–23] In this study, 4% of adult geese (n = 6) had WBC counts above the 95% confidence interval, and no geese had WBCs that fell below this confidence interval. The small percentage of birds with WBC counts that fell below the published reference intervals were most likely representative of a normal distribution in this group of birds and may not be clinically relevant. The high WBC counts may indicate some level of chronic stress or, more likely, inflammation (eg, from disease or injury). Even though no apparent health problems were noted on physical examination, underlying infectious diseases such as aspergillosis, mycobacteriosis, or other bacterial infections cannot be excluded in birds with leukocytosis.[24]

The estimated WBC count method performed in this study was chosen because it allowed samples to be collected in a multi-study field setting and stored for later analysis in the lab.[1,2,20] Quantitative methods (Eopette, Exotic Animal Solutions, LLC, Hueytown, AL, USA, http://www.exoticanimalsolutions.com/Eopette-Test-kit-0001.htm and the Natt and Herrick stain method) use whole anticoagulated blood diluted with respective solutions, and samples must be analyzed with a hemocytometer within minutes of preparation.[25] By comparison, dried blood smears were easily prepared on site, and the slides were stained and examined at a later time.[24] We recognize the potential inaccuracy of estimated WBC counts compared to quantitative counts, but this technique is still widely used in avian practice.[26–28] Moreover, during this study, a single experienced individual analyzed all slides by using a consistent technique.

The mean heterophil percentages detected in the Canada geese in North Carolina were lower than those referenced in the literature.[1,23] In mallard ducks (*Anas platyrhnchos*), the percentage of heterophils decreased during and immediately following a remige molt.[29] Both adult and juvenile Canada geese were molting during the current study, which may account for the lower heterophil percentage observed.

Anseriformes, including Canada geese, usually have a higher relative number of circulating lymphocytes than heterophils with an H : L ratio between 0.4 : 1 and 0.7 : 1, and juvenile birds often have higher lymphocyte percentages than adults.[23,24] In the current study, juvenile geese had higher lymphocyte and lower heterophil percentages than adult geese, which resulted in lower H : L ratios. The average H : L ratios in the current study were lower than those reported for other anseriform species, which may be a combination of sampling juveniles and the molting status of all geese. H : L ratios can be used as an indicator of stress in birds.[30] In waterfowl, the response to stress and inflammation is initial leukopenia followed by a stress leukogram (ie, leukocytosis, with heterophilia and lymphopenia resulting in an increased H : L ratio).[23,24] Therefore, most birds in this study did not appear to exhibit a stress leukogram, because the mean estimated WBC

counts were within reference intervals and the H : L ratios were below reported intervals.[21,23,31]

Occasionally, reactive lymphocytes are detected in normal birds. However, large numbers of reactive lymphocytes may be associated with antigenic stimulation related to infectious disease.[24,32] In this study, lymphocytes containing pseudopods, split or irregular nuclei, and/or purple granules were considered reactive.[23] There could be a potential relationship between reactive lymphocytes and some type of infectious process (eg, gastrointestinal parasites, bacterial enteritis, aspergillosis, etc) in some birds with elevated WBC counts. In birds with normal hematologic parameters, morphologically variable lymphocytes may be within normal limits.[24]

Compared to other published results,[1,11,20,23] the increased percentage of eosinophils was unexpected. The characteristic eosinophil percentages in this study in a presumed normal free-ranging population may have been a response to parasitism, specifically involving gastrointestinal parasites,[21,33,34] or exposure to foreign antigens in a delayed hypersensitivity reaction,[22,24] (eg, involving an acute infection with a *Mycoplasma* species).[35] Although internal and external parasite counts were not determined in our study, parasitemia is a potential explanation for the higher eosinophil percentage present in free-ranging geese.

The literature reference intervals for monocytes and basophils vary; therefore, we were unable to determine if the values obtained in this study were relatively high or low.[1,11,23] Basophilia in birds has been associated with physiologic stress and forced molting,[28,32] but the function is not fully understood.[22] Basophilia has also been detected in birds with respiratory disease, bacterial infections, acute inflammation, and internal and external parasitism with or without eosinophilia.[28] Monocytosis is seen in cases of acute and chronic infection and inflammation, especially involving bacterial and fungal diseases, along with parasitism and zinc-deficient diets.[22,28,32] Birds with chronic infectious diseases are typically emaciated, but the birds in this study were well-fleshed and lacked apparent health abnormalities. Monocytes can be difficult to distinguish from large lymphocytes, which could account for the high variability of values for monocytes reported in the literature.[28,32] Although great care was taken to distinguish different cell types, it was possible to misidentify lymphocytes as monocytes in this study, resulting in a higher monocyte percentage.

This study may have underestimated the true number of resident Canada geese infected with blood parasites, because low parasite burdens or early infections could have been missed during direct microscopic examination. Traditional microscopy methods were used to detect the parasite in peripheral blood smears, which have recently been shown to be similar to, or less sensitive than polymerase chain reaction–based methods.[36] In the case of *Hemoproteus,* only the gametocyte stage is detected in peripheral blood; therefore, birds with an early infection could be incorrectly identified as negative.[22] This is especially relevant to the smaller number of juveniles in this study who may have been exposed in their first year of life when the parasite had not yet entered their peripheral RBCs. Previous studies have reported an increased prevalence of *Hemoproteus* with increasing age in waterfowl, which agrees with the results of this study.[37,38]

Hemoproteus is relatively common in many species of wild birds and is usually nonpathogenic.[24,39] Birds that are immunocompromised, whether from concurrent disease or traumatic injury, may show clinical signs of disease from these hemoparasites.[22] In general, the hematologic changes seen with the erythrocyte form of infection include leukocytosis and anemia.[40] As the immune status of the bird increases, the degree of parasitemia has been shown to decrease.[22,36] In this study, the goose with the highest parasite count had a leukocytosis and eosinophilia whereas the other 3 *Hemoproteus*-positive geese with low parasite burdens had a relatively normal hemogram.

This study provided a unique opportunity to evaluate hematologic parameters and hemoparasites of adult and juvenile resident Canada geese. The large number of samples provided a good baseline for blood reference intervals from an apparently healthy resident Canada goose population from North Carolina and may be useful for evaluating physiologic status of Canada geese in an ecological, rehabilitative, or captive setting.

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