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Blood Parasites of Blue-winged Teal (*Anas discors*) from Two Migratory Corridors, in the Southern USA

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ABSTRACT: We collected 180 Blue-winged Teal (*Anas discors*) in September and October 2002 from Florida, US (n=100, representing the eastern migratory corridor) and the Louisiana-Texas, US, border (n=80, representing the western migratory corridor) and examined for blood parasites using thin heart-blood smears. *Leucocytozoon simondi*, *Haemoproteus nettionis*, and microfilariae were found in 16, 23, and 27 birds, respectively. Prevalence of *L. simondi* and *H. nettionis* did not vary by migratory corridor, but the prevalence of microfilariae was higher in the western corridor (23%) than the eastern corridor (9%). No differences in prevalence of *L. simondi*, *H. nettionis*, and microfilariae were observed by host age or sex. The mean density of *L. simondi* and *H. nettionis* averaged 1.5±0.3 and 2.3±0.4 (±SE per 3,000 erythrocytes), respectively. Ranked abundance models for main and interactive effects of corridor, age, and sex were not statistically significant for *L. simondi* or *H. nettionis*. Low prevalence and abundance of hematozoa in early autumn migrants reflects the likelihood of low exposure probabilities of Blue-winged Teal on the breeding grounds, compared to their congeners.

Key words: *Anas discors*, blood parasites, Blue-winged Teal, *Haemoproteus nettionis*, *Leucocytozoon simondi*, microfilariae.

Hematozoans in avian hosts have the potential to cause disease (Valkiunas 2005). Migratory avian hosts are exposed to more hematozoan species, have higher risk of infection than sedentary hosts (Figuerola and Green 2000), and transport parasites between breeding and wintering areas and along their migratory routes (Fedynich et al. 1993; Smith and Ramey 2015). The Blue-winged Teal (*Anas discors*) provides the opportunity to examine a host-parasite system in which the host is a common breeding duck across the northeastern half of North America, migrates in early autumn (begins August), and migrates transnationally and transcontinentally (Rohwer et al. 2002). Blue-winged Teal primarily use an eastern migratory corridor and a western corridor in which those migrating along the eastern corridor move from northeastern breeding areas in North America to Florida, US, and continue southward into Guyana, Colombia, and Brazil, whereas those using the western corridor migrate from Saskatchewan, Canada, and follow the Mississippi River Valley to the border of Texas and Louisiana, US, ending in Mexico and Central and South America (Bellrose 1980). Herein we quantify prevalence and abundance of blood parasites in Blue-winged Teal using thin blood smears and determine if these values vary by migratory corridor, host age, and host sex.

We collected 180 Blue-winged Teal between 21 September and 30 October 2002. These included 100 (34 adult males, 10 adult females, 14 juvenile males, 42 juvenile females) from Glades, Martin, Palm Beach, and Okeechobee counties, Florida, US, representing the eastern corridor (28.35′–28.18′N, 80.49′–81.24′W), and 80 (21 adult males, 13 adult females, 21 juvenile males, 25 juvenile females) from Jasper, Jefferson, Newton, Orange, Sabine, and San Augustine counties in Texas, and Beauregard, Calcasieu, Cameron, Sabine, and Vernon parishes in Louisiana, representing the western corridor (29°53′–30°12′N, 93°12′–93°56′W).

Blue-winged Teal were collected by shotgun in accordance with state and federal permits (Florida: WX02177; Louisiana: LNHP-02-107; Texas: SPR-0602-222; US Fish and Wildlife Service: MB056380-0). The study was approved by the Texas A&M University–Kingsville, Kingsville, Texas, Institutional Animal Care and Use Committee (Approval 2002-8-15). Definitions follow Bush et al. (1997) where prevalence is the number of hosts infected with a particular parasite species divided by the number of hosts examined (expressed as a percentage) and density is the number of erythrocytes infected with a particular protozoan species expressed per the total number of erythrocytes examined from an infected host. Mean abundance is defined as the total number of host blood cells infected with a particular protozoan species divided by the number of erythrocytes examined, and this quotient was divided by total number of hosts examined (expressed as a proportion).

Within 10 min of bird death, heart blood (approximately 0.1 mL) was extracted using a pipette from which two thin smears were made on microscope slides, air dried, fixed in 95% methanol for 1 min, and stained using Diff-Quik® (Dade Behring, Deerfield, Illinois, USA). To determine parasite prevalence, each slide was scanned for 15 min (30 min per bird) with a microscope at 1,000× magnification. Each slide was examined for an additional 5 min at 400× magnification (10 min per bird).

Density estimation followed procedures recommended by Godfrey et al. (1987) in which a positive smear is viewed at 1,000× magnification and, using a Miller ocular disc, 30 replicates of 100 erythrocytes each are counted, and the number of parasites of each species recorded. To decrease the chance of bias, random numbers were used to determine the order in which the slides were counted and the distance between locations viewed in each cardinal direction on the slide. Although the protocols of Godfrey et al. (1987) were developed for *Haemoproteus* spp., they have been successfully applied to *Leucocytozoon* spp. (Fedynich and Rhodes 1995; DeJong et al. 2001). No satisfactory techniques are available to estimate density of microfilariae using blood smears; consequently, only prevalence data are reported for microfilariae. In instances where protozoans were found in initial prevalence screening, but density could not be quantified (i.e., <1 per 3,000 erythrocytes counted), a value of 0.5 was assigned to the infected host (=0.5 parasites/3,000 cells counted), which allows their inclusion in summary statistics and statistical analyses of small samples (Clarke 1998).

Chi-square 2×2 contingency-table analysis was used to determine if the prevalence varied by the main effects of migratory corridor, host age, and host sex. Abundance data were rank transformed, and analysis of variance was used to examine the main and interaction effects variables of migratory corridor, host age, and host sex. Voucher specimens of *Haemoproteus nettionis* (SHSUP001579) and *Leucocytozoon simondi* (SHSUP001578) were deposited into the Sam Houston State University Parasite Collection, Huntsville, Texas, US.

Fifty-four (30%) Blue-winged Teal demonstrated infections in blood smears. *Leucocytozoon simondi* was found in 16 (9%) birds, *H. nettionis* was found in 23 (13%) birds, and microfilariae were found in 27 (15%) birds (Table 1). No *Plasmodium* spp. were observed. Multiple infections occurred in 10 Blue-winged Teal. Of these, three birds were observed with *L. simondi* and microfilariae, one bird with *H. nettionis* and microfilariae, four birds with *L. simondi* and *H. nettionis*, and two birds with *L. simondi*, *H. nettionis*, and microfilariae.

Prevalence of *L. simondi* and *H. nettionis* did not differ (P=0.64 and P=0.06) between migratory corridors, whereas the prevalence of microfilariae was higher (P=0.01) in the western corridor (23%) than the eastern corridor (9%). No differences in prevalence of *L. simondi*, *H. nettionis*, or microfilariae were observed between host ages (P=0.57, P=0.99, P=0.47, respectively) or between host sexes (P=0.60, P=0.50, P=0.53, respectively).

Densities of *L. simondi* and *H. nettionis* averaged 1.5±0.3 ($\bar{x}$±SE) per 3,000 erythrocytes and 2.3±0.4 per 3,000 erythrocytes, respectively. Mean abundance of *L. simondi*

TABLE 1. Prevalence (%) and 95% confidence interval (in parentheses) of *Leucocytozoon simondi*, *Haemoproteus nettionis*, and microfilaria by migratory corridor, host age, and host sex observed in blood smears of Blue-winged Teal (*Anas discors*) collected in Florida (eastern corridor) and Texas and Louisiana (western corridor), USA, during autumn 2002.

	Migratory corridor		Host age		Host sex		
Parasite species	Eastern n=100	Western n=80	Juvenile n=102	Adult n=78	Male n=90	Female n=90	Total n=180
Leucocytozoon simondi	8 (3.5–15.2)	10 (4.4–18.9)	8 (3.4–14.9)	10 (4.5–19.2)	8 (3.2–15.4)	10 (4.7–18.1)	9 (5.2–14.0)
Haemoproteus nettionis	17 (10.2–25.8)	8 (2.8–15.6)	13 (7.0–20.8)	13 (6.3–22.3)	14 (7.9–23.4)	11 (5.5–19.5)	13 (8.3–18.5)
Microfilaria	9 (4.2–16.4)	23 (13.9–33.2)	17 (10.0–25.3)	13 (6.3–22.3)	13 (7.1–22.1)	17 (9.6–26.0)	15 (10.1–21.1)

and *H. nettionis* was 0.1±<0.1 and 0.3±0.1, respectively (Table 2). The overall analysis of variance models for rank mean abundance of *L. simondi* and *H. nettionis* were not significant (P=0.96 and P=0.40, respectively) and were not further considered for main and interaction effects variables.

Numerous studies have examined blood parasites in North American waterfowl in which hematozoan prevalence can differ across various extrinsic and intrinsic variables (Bennett et al. 1975, 1982; Greiner et al. 1975; Loven et al. 1980). However, fewer waterfowl studies have examined these variables within the context of hematozoan density or abundance (Fedynich et al. 1993; DeJong and Muzzall 2000; DeJong et al. 2001). *Haemoproteus nettionis* was the most prevalent and abundant blood protozoan found in our study. However, both *L. simondi* and *H. nettionis* had relatively low prevalence values (5.2–18.5%) and abundance values (0.1–0.3 parasites per 3,000 erythrocytes) overall, which was reflected across migratory corridor, host age, and host sex variables. The low levels likely suggest a strategy by hematozoa not to produce high numbers of gametocytes during periods when vector populations are declining or minimal (i.e., autumn and winter) (Allan and Mahart 1989). Alternatively, because Blue-winged Teal are the last to arrive on breeding grounds and first to migrate in autumn, the probability of infection on the breeding grounds may be lower than for species that arrive earlier or depart later than Blue-winged Teal. This is evident by a tendency for infections (as measured by prevalence) to be lower in Blue-winged Teal than infections found within their congenerics during the same season at the same geographic locations (>50 host individuals of same species sampled; Bennett et al. 1974, 1975, 1982; Kocan et al. 1979).

No *Plasmodium* spp. were detected in this study. It is possible that they may have been present in extremely low densities where PCR

TABLE 2. Abundance (mean number of individual parasites per 3,000 erythrocytes/hosts sampled±SE) values and range of *Leucocytozoon simondi* and *Haemoproteus nettionis* by migratory corridor, host age, and host sex observed in blood smears of Blue-winged Teal (*Anas discors*) collected in Florida (eastern corridor) and Texas and Louisiana (western corridor), USA, during autumn 2002.

	Migratory corridor		Host age		Host sex		
Parasite species	Eastern $\bar{x}$±SE	Western $\bar{x}$±SE	Juvenile $\bar{x}$±SE	Adult $\bar{x}$±SE	Male $\bar{x}$±SE	Female $\bar{x}$±SE	Total $\bar{x}$±SE
Leucocytozoon simondi	0.1±<0.1	0.2±0.1	0.1±0.1	0.2±0.1	0.1±<0.1	0.2±0.1	0.1±<0.1
Haemoproteus nettionis	0.4±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1

would have been able to detect their presence better than microscopy (Garamszegi 2010). Microscopy may underestimate prevalence, and PCR focused on one group (i.e., protozoa) may not detect another (i.e., microfilaria). Some studies using comparative techniques have shown both methods produced generally similar results for haemosporidians (Valkiunas et al. 2008; Garamszegi 2010), whereas others found mixed results (Krams et al. 2012). Additionally, quantification of parasitemia can be achieved by either light microscopy or quantitative PCR with suitably similar results in general although qPCR can give better resolution (Biedrzycka et al. 2015). A combination of both techniques would be ideal for future studies.

We characterized infections of *L. simondi*, *H. nettionis*, and microfilaria in a migrating population of Blue-winged Teal. Based on our findings, the low prevalence and abundance of hematozoa in these early autumn migrants reflects the likelihood of low exposure probabilities of Blue-winged Teal on the breeding grounds, compared to their congeners.

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