WDA
Wildlife Disease Association
Founded in 1951

JOURNAL OF WILDLIFE DISEASES

VOLUME 52 NUMBER 3 JULY 2016

Hoberg EP, Polley L, Gunn A, Nishi JS. 1995. *Umingmakstrongylus pallikuukensis* gen. nov. et sp. nov. (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. *Can J Zool* 73:2266–2282.

Ingebjørg HN, Kimberlee B, Jacques G. 2016. Anti-*brucella* antibodies in moose (*Alces alces gigas*), muskoxen (*Ovibos moschatus*), and plains bison (*Bison bison bison*) in Alaska, USA. *J Wildl Dis* 52: 96–99.

Inoshima Y, Morooka A, Sentsui H. 2000. Detection and diagnosis of parapoxvirus by the polymerase chain reaction. *J Virol Methods* 84:201–208.

Kafle P, Lejeune M, Verocai GG, Hoberg EP, Kutz SJ. 2015. Morphological and morphometric differentiation of dorsal-spined first stage larvae of lungworms (Nematoda: Protostrongylidae) infecting muskoxen (*Ovibos moschatus*) in the central Canadian Arctic. *Int J Parasitol Parasites Wildl* 4:283–290.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.

Kutz SJ, Bollinger T, Branigan M, Checkley S, Davison T, Dumond M, Elkin B, Forde T, Hutchins W, Niptanatiak A, et al. 2015. *Erysipelothrix rhusiopathiae* associated with recent widespread muskox mortalities in the Canadian Arctic. *Can Vet J* 56: 560–563.

Kutz SJ, Checkley S, Verocai GG, Dumond M, Hoberg EP, Peacock R, Wu JP, Orsel K, Seegers K, Warren AL, et al. 2013. Invasion, establishment, and range expansion of two parasitic nematodes in the Canadian Arctic. *Glob Change Biol* 19:3254–3262.

Lopez-Goni I, Garcia-Yoldi D, Marin CM, de Miguel MJ, Barquero-Calvo E, Guzman-Verri C, Albert D, Garin-Bastuji B. 2011. New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. *Vet Microbiol* 154:152–155.

Nielsen K, Smith P, Yu W, Nicoletti P, Elzer P, Vigliocco A, Silva P, Bermudez R, Renteria T, Moreno F, et al. 2004. Enzyme immunoassay for the diagnosis of brucellosis: Chimeric Protein A–Protein G as a common enzyme labeled detection reagent for sera for different animal species. *Vet Microbiol* 101:123–129.

Post E, Bhatt US, Bitz CM, Brodie JF, Fulton TL, Hebblewhite M, Kerby J, Kutz SJ, Stirling I, Walker DA. 2013. Ecological consequences of sea-ice decline. *Science* 341:519–524.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.

Verocai GG, Kutz SJ, Simard M, Hoberg EP. 2014. *Varestrongylus eleguneniensis* sp. n. (Nematoda: Protostrongylidae): A widespread, multi-host lungworm of wild North American ungulates, with an emended diagnosis for the genus and explorations of biogeography. *Parasit Vectors* 7:556.

Vikøren T, Lillehaug A, Åkerstedt J, Bretten T, Haugum M, Tryland M. 2008. A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (*Ovibos moschatus*) population in Norway. *Vet Microbiol* 127: 10–20.

Ytrehus B, Davidson RK, Isaksen K. 2015. Single causative factor for severe pneumonia epizootics in muskoxen? *Ecohealth* 12:395–397.

Zarnke RL, Dieterich RA, Neiland KA, Ranglack G. 1983. Serologic and experimental investigations of contagious ecthyma in Alaska. *J Wildl Dis* 19:170–174.

Submitted for publication 3 December 2015.
Accepted 10 March 2016.

DOI: 10.7589/2016-01-010

Journal of Wildlife Diseases, 52(3), 2016, pp. 725–729

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

Jason M. Garvon,[1,4,5] Joanna B. Mott,[2,3] Sandy Serio Jacobs,[2] and Alan M. Fedynich[1] [1]Caesar Kleberg Wildlife Research Institute, Texas A&M University–Kingsville, 700 University Blvd., MSC 218, Kingsville, Texas 78363, USA; [2]Department of Physical and Life Sciences, Texas A&M University–Corpus Christi, 6300 Ocean Drive, Unit 5802, Corpus Christi, Texas 78412, USA; [3]Current address: Department of Biology, 951 Carrier Drive, MSC 7801, James Madison University, Harrisonburg, Virginia 22807, USA; [4]Current address: Department of Biology, Lake Superior State University, 650 W Easterday Avenue, Sault Ste. Marie, Michigan 49783, USA; [5]Corresponding author (email: jgarvon@lssu.edu)

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* 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 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)

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.

We acknowledge the assistance in field collections by Paul Gray, Ross Freeman, Bill Freeman, Perry Smith, Trey Pearson, and Marc Epstein, and thank the staff of the Cameron Prairie National Wildlife Refuge, J. D. Murphree Wildlife Management Area, Merritt Island National Wildlife Refuge, and T. M. Goodwin Waterfowl Management Unit. We also thank the many landowners and lease holders for allowing property access. This is manuscript 16-113 of the Caesar Kleberg Wildlife Research Institute.

LITERATURE CITED

Allan RA, Mahrt JL. 1989. Influence of transmission period on primary and relapse patterns of infection of *Leucocytozoon* spp. and *Haemoproteus mansoni*. *Am Midl Nat* 121:341–349.

Bellrose FC. 1980. Ducks, geese and swans of North America. Stackpole Books, Harrisburg, Pennsylvania, 540 pp.

Bennett GF, Blandin W, Heusmann HW, Campbell AG. 1974. Hematozoa of the Anatidae of the Atlantic Flyway. I. Massachusetts. *J Wildl Dis* 10:442–451.

Bennett GF, Nieman DJ, Turner B, Kuyt E, Whiteway M, Greiner EC. 1982. Blood parasites of prairie anatids and their implication in waterfowl management in Alberta and Saskatchewan. *J Wildl Dis* 18:287–296.

Bennett GF, Smith AD, Whitman W, Cameron M. 1975. Hematozoa of the Anatidae of the Atlantic Flyway. II. The maritime provinces of Canada. *J Wildl Dis* 11: 280–289.

Biedrzycka A, Migalska M, Bielański W. 2015. A quantitative PCR protocol for detecting specific *Haemoproteus* lineages: Molecular characterization of blood parasites in a sedge warbler population from southern Poland. *J Ornithol* 156:201–208.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al revisited. *J Parasitol* 83:575–583.

Clarke JU. 1998. Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. *Environ Sci Technol* 32:177–183.

DeJong RJ, Muzzall PM. 2000. Hematozoa of waterfowl from Michigan. *J Wildl Dis* 36:767–773.

DeJong RJ, Reimink RL, Blankespoor HD. 2001. Hematozoa of hatch-year Common Mergansers from Michigan. *J Wildl Dis* 37:403–407.

Fedynich AM, Pence DB, Godfrey RD Jr. 1993. Hemosporids (Apicomplexa, Hematozoea, Hemosporida) of anatids from the Southern High Plains of Texas. *J Helminthol Soc Wash* 60:35–38.

Fedynich AM, Rhodes OE Jr. 1995. Hemosporid (Apicomplexa, Hematozoea, Hemosporida) community structure and pattern in wintering Wild Turkeys. *J Wildl Dis* 31:404–409.

Figuerola J, Green AJ. 2000. Hematozoan parasites and migratory behaviour in waterfowl. *Evol Ecol* 14:143–153.

Garamszegi LZ. 2010. The sensitivity of microscopy and PCR-based detection methods affecting estimates of prevalence of blood parasites in birds. *J Parasitol* 96: 1197–1203.

Godfrey RD Jr, Fedynich AM, Pence DB. 1987. Quantification of hematozoa in blood smears. *J Wildl Dis* 23:558–565.

Greiner EC, Bennett GF, White EM, Coombs RF. 1975. Distribution of the avian hematozoa of North America. *Can J Zool* 53:1762–1787.

Kocan AA, Shaw MG, Morgan PM. 1979. Some parasitic and infectious diseases in waterfowl in Oklahoma. *J Wildl Dis* 15:137–141.

Krams I, Suraka V, Cīrule D, Hukkanen M, Tummeleht L, Mierauskas P, Rytkonen S, Rantala MJ, Vrublevska J, Orell M, et al. 2012. A comparison of microscopy and PCR diagnostics for low intensity infections of haemosporidian parasites in the Siberian Tit *Poecile cinctus*. *Ann Zool Fennici* 49:331–340.

Loven JS, Bolen EG, Cain BW. 1980. Blood parasitemia in a south Texas wintering waterfowl population. *J Wildl Dis* 16:25–28.

Rohwer FC, Johnson WP, Loos ER. 2002. Blue-winged Teal (*Anas discors*). In: *The birds of North America, No. 625*, Poole A, Gill F, editors. The Birds of North America, Philadelphia, Pennsylvania, 35 pp.

Smith MM, Ramey AM. 2015. Prevalence and genetic diversity of hematazoa in South American waterfowl and evidence for intercontinental redistribution of parasites by migratory birds. *Int J Parasitol Parasites Wildl* 4:22–28.

Valkiunas G. 2005. *Avian malaria parasites and other haemosporidia*. CRC Press, Boca Raton, Florida, 932 pp.

Valkiunas G, Iezhova TA, Krizanauskiene A, Palinauskas V, Sehgal RNM, Bensch S. 2008. A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *J Parasitol* 94:1395–1401.

Submitted for publication 12 January 2016.
Accepted 10 March 2016.