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Authors: Valkiūnas, Gediminas, Iezhova, Tatjana A., Brooks, Daniel R., Hanelt, Ben, Brant, Sara V., et al.

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Gediminas Valkiūnas,[1,6] **Tatjana A. Iezhova,**[1] **Daniel R. Brooks,**[2] **Ben Hanelt,**[3,4] **Sara V. Brant,**[3,4] **Marie E. Sutherlin,**[3,5] **and Douglas Causey**[5] [1] Institute of Ecology, Vilnius University, Akademijos 2, Vilnius-21, LT-08412, Lithuania; [2] Department of Zoology, University of Toronto, Toronto, Ontario M5S 3G5, Canada; [3] School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588, USA; [4] Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA; [5] Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, USA; [6] Corresponding author (email: gedvalk@ekoi.lt)

ABSTRACT: Birds from the Area de Conservación Guanacaste in northwestern Costa Rica were surveyed for blood parasites in June 2001 and December 2001–January 2002. Of 354 birds examined, representing 141 species of 35 families and 15 orders, 44 (12.4%) were infected with blood parasites. Species of *Haemoproteus* (4.8% prevalence), *Plasmodium* (0.6%), *Leucocytozoon* (0.3%), *Trypanosoma* (2.0%), and microfilariae (7.6%) were recorded. Twelve species of birds in this survey were examined for blood parasites for the first time. Several new host-parasite associations were identified.

Key words: Avian hematozoa, Costa Rica, *Haemoproteus, Leucocytozoon,* microfilariae, *Plasmodium, Trypanosoma*.

As part of a comprehensive inventory of eukaryotic parasites of vertebrates being conducted in the Area de Conservación Guanacaste (ACG) in northwestern Costa Rica, we investigated blood parasites of birds found in diverse habitats including the tropical dry forest and lower-, mid-, and upper canopy rainforests. Only one study has reported the hematozoa of birds in Costa Rica (Young et al., 1993), and it was focused on lower-canopy species in the Monteverde rainforest. In the present article, the parasite fauna, prevalences, intensities, and new host-parasite associations are described. These data are discussed relative to similar parasites recorded from other areas, with the identification of some promising directions for future fieldwork research into epizootiology of avian hematozoan infections.

The ACG occupies the narrow Pacific dry forest-covered coastal plain and a 300-m volcanic plateau that extends 10–20 km inland and rises to three cloud forest–covered volcanoes and then drops down to the Atlantic rainforest-covered coastal plain, passing through six Holdridge Life Zones in the process. This conserved wild land is 85 km long and 15–25 km wide. The ACG has been estimated to contain 65% of Costa Rica's biota, or 235,000 species (Janzen, 1996), as many are in the continental United States and Canada. The ACG climate is a complex mosaic of seasonality, ranging from a 6-mo dry season at the western end (complete with cacti and dwarf forest), to virtually no seasonality in the extremely wet and almost permanent clouds on top of volcanoes, to the 1–4 mo semidry season of the Atlantic rainforest.

Three hundred and fifty-four birds belonging to 141 species, 35 families, and 15 orders from 31 sites, located between 10°49′N and 10°57′N, 85°23′W and 85°38′W and 205–680 m above sea level in the ACG, were collected during June 2001 (wet season) and December 2001–January 2002 (dry season) (Table 1). For details and exact localities, see http://brooksweb.zoo.utoronto.ca/index.html. Only 13 of the species collected were winter migrants from North America. The remaining 128 species of birds were resident year-round in the ACG. Host specimens were collected by mist net or firearm under the authority of CITES Permits US9258251 and CR9123440, Costa Rica Ministero del Ambiente y Energia Licencia 203640283; Resoluciones 215-2001-OFAU and 411-2001-OFAU; Harvard University IACUC Protocol 21-09; and US Department of Agriculture APHIS permit 47956 (form VS16-6A). Approximately 0.2 ml of blood was collected by sterile pipette from the lateral tibiotarsal venal sinus in birds before or immediately after they

TABLE 1. Occurrence of hematozoa in birds from Costa Rica, 2001–02.

Bird species and family	No. examined	No. infected	Parasite present[a]				
			H	P	L	T	M
Ciconiidae							
Mycteria americana	1	1	1				
Columbidae							
Columba speciosa	1	1	1				
Columbina inca[b]	2	1	1[c]				
Columbina passerina	1	1	1				
Corvidae							
Calocitta formosa	2	2					2
Cracidae							
Crax rubra	1	1	1[c]			1[c]	
Cuculidae							
Piaya cayana	2	2					2
Emberizidae							
Arremon aurantiirostris	6	1					1[c]
Arremonops conirostris	5	1					1
Cyanocompsa cyanoides	3	1					1
Formicariidae							
Gymnocichla nudiceps	1	1				1	
Thamnophilus doliatus	5	1				1	1[c]
Galbulidae							
Galbula ruficauda	1	1				1[c]	
Icteridae							
Icterus pustulatus	1	1					1
Quiscalus mexicanus	1	1					1
Parulidae							
Basileuterus rufifrons	14	1					1[c]
Phaeothlypis fulvicauda	3	1	1[c]				1[c]
Vermivora peregrina[d]	6	1	1				
Sylviidae							
Polioptila albiloris	2	1					1[c]
Thraupidae							
Chlorophanes spiza	2	1	1[c]				
Habia rubica	1	1					1
Piranga ludoviciana[d]	1	1	1	1		1	1
Tangara icterocephala	2	1	1				
Trochilidae							
Amazilia rutila	9	1					1[c]
Amazilia saucerrotei[b]	20	1	1[c]				
Archilochus colubris[d]	1	1	1				
Troglodytidae							
Cyphorhinus phaeocephalus	2	1	1[c]				
Trogonidae							
Trogon violaceus	2	1					1
Turdidae							
Hylocichla mustelina[d]	4	2			1		1
Turdus assimilis	4	1					1

TABLE 1.   Continued

Bird species and family	No. examined	No. infected	Parasite present[a]				
			H	P	L	T	M
Turdus grayi	3	1					1
Tyrannidae							
Megarynchus pitangua	2	1	1				1
Myiodynastes luteiventris	1	1	1				
Myiozetetes similis	1	1					1
Pachyramphus cinnamomeus	2	2				2	2
Rhytipterna holerythra	1	1					1[c]
Vireonidae							
Hylophilus decurtatus	2	2	1[c]	1[c]			
Vireo flavoviridis	6	2	1				2
Uninfected species[e]	230						
Totals	354	44	17	2	1	7	27
Prevalence, %		12.4	4.8	0.6	0.3	2.0	7.6

[a] No. of birds with each parasite. H = *Haemoproteus* sp.; P = *Plasmodium* sp.; L = *Leucocytozoon* sp.; T = *Trypanosoma* sp.; M = Microfilaria.

[b] First time species examined for blood parasites.

[c] New host record.

[d] North American migrant species.

[e] Uninfected species (no. of individuals examined): Accipitridae: *Buteo magnirostris* (4), *Buteogalus anthracinus* (2), *Leucopternis albicollis* (1); Alcedinidae: *Chloroceryle americana* (1); Anatidae: *Dendrocygna autumnalis* (1); Burhinidae: *Burhinus bistriatus* (1); Caprimulgidae: *Nyctidromus albicollis* (3); Columbidae: *Geotrygon montana* (1), *Leptotila cassini* (1), *L. verreauxi* (2); Cracidae: *Penelope purpurascens* (1); Cuculidae: *Crotophaga sulcirostris* (1); Dendrocolaptidae: *Dendrocincla fuliginosa* (1), *D. homochroa* (3), *Dendrocolaptes certhia* (2), *Glyphorynchus spirurus* (1), *Lepidocolaptes souleyetii* (1), *Sittasomus griseicapillus* (3), *Xiphorhynchus erythropygius* (1); Emberizidae: *Aimophila ruficauda*[b] (1), *Arremonops rufivirgatus* (5), *Oryzoborus funereus* (3), *Saltator maximus* (6), *Sporophila aurita* (5), *Tiaris olivacea* (2), *Volatinia jacarina* (1); Falconidae: *Falco rufigularis* (1), *Herpetotheres cachinnans* (1); Formicariidae: *Dysithamnus striaticeps*[b] (1), *Formicarius analis* (1), *Gymnopithys leucaspis* (4), *Hylophylax naevioides* (10), *Thamnophilus punctatus* (1); Furnariidae: *Automolus ochrolaemus* (7), *A. rubiginosus* (1), *Phaenostictus mcleannani* (2), *Sclerurus guatemalensis* (1), *Syndactyla subalaris* (1), *Xenops minutus* (2); Icteridae: *Icterus galbula*[d] (1); Momotidae: *Momotus momota* (1); Parulidae: *Dendroica pensylvanica*[d] (1), *Geothlypis poliocephala* (2), *Mniotilta varia*[d] (1), *Oporornis formosus*[d] (2), *Seiurus aurocapilla*[d] (2), *S. motacilla*[d] (1), *S. noveboracensis*[d] (2); Pelecanidae: *Pelecanus occidentalis* (1); Picidae: *Campephilus guatemalensis*[b] (1); Pipridae: *Chiroxiphia linearis* (10), *Manacus candei* (5), *Pipra coronata* (1); Psittacidae: *Brotogeris jugularis* (2); Ramphastidae: *Ramphastos sulfuratus* (1); Sylviidae: *Polioptila plumbea* (1), *Ramphocaenus melanurus*[b] (1); Thraupidae: *Chlorothraupis carmioli*[b] (7), *Euphonia affinis* (1), *E. anneae* (1), *E. gouldi* (1), *Piranga flava* (1), *P. rubra*[d] (2), *Ramphocelus passerinii* (7), *Tachyphonus luctuosus* (2), *Tangara larvata* (2), *Thraupis episcopus* (3); Tinamidae: *Crypturellus cinnamomeus* (1); Trochilidae: *Amazilia tzacatl* (4), *Eupherusa eximia*[b] (2), *Florisuga mellivora* (1), *Heliomaster constantii*[b] (2), *Heliothryx barroti* (1), *Phaethornis guy* (3), *P. longuemareus* (3), *P. superciliosus* (2), *Thalurania columbica*[b] (1), *Threnetes ruckeri* (2); Troglodytidae: *Campylorhynchus rufinucha* (1), *Henicorhina leucosticta* (6), *Thryothorus modestus* (1), *T. nigricapillus* (4), *T. pleurostictus* (6); Trogonidae: *Trogon elegans* (1), *T. massena* (1); Tyrannidae: *Attila spadiceus* (3), *Contopus cinereus* (2), *Elaenia flavogaster* (1), *Empidonax* sp. (1), *Leptogon amaurocephalus* (1), *Lophotriccus pileatus* (2), *Mionectes oleagineus* (5), *M. olivaceus* (1), *Myiarchus nuttingi*[b] (1), *M. tuberculifer* (1), *M. tyrannulus* (7), *Myiozetetes granadensis* (1), *Todirostrum cinereum* (1), *T. sylvia* (1), *Tolmomyias sulphurescens* (5), *Tyrannus forficatus*[b,d] (1), *Zimmerius vilissimus* (1); Vireonidae: *Hylophilus ochraceiceps* (3).

were killed. Thin blood films were airdried, fixed in ethanol within 2 hr of collection in the field, and then stained with Giemsa in the laboratory. For each slide, approximately 120–150 fields were examined at low magnification (400×), and then at least 100 fields were studied at high magnification (1,000×). The intensity of infection was estimated as a percentage by actual counting the number of parasites per 1,000 red blood cells or per 10,000 red blood cells if infections were light (i.e., <0.1%), as recommended by Godfrey et al. (1987). Blood parasites were identified according to the methods of Baker (1976) and Valkiūnas (1997), in which illustrations of all parasites mentioned in the present article are given. Prevalences were com-

pared by Yates-corrected χ^2 test (Wilkinson, 1989). A *P* value ≤0.05 was considered to be significant. Representative blood slides were deposited in the Institute of Ecology, Vilnius University, Vilnius, Lithuania, with accession numbers 01-4, 01-31, 01-135, 01-360, 01-366, 02-073, 02-075, 02-093, 02-142, 02-146, 02-148, and 02-195, Series MCZ, Costa Rica.

Prevalences of blood parasites in the study were low (Table 1). Only 44 birds (12% overall prevalence) of 38 species (27% of examined species) were found to harbor hematozoa. Species of *Haemoproteus, Plasmodium, Leucocytozoon, Trypanosoma,* and microfilariae of filariid nematodes were recorded. The majority of the infections (86% of all positive birds) were single infections. There was no significant difference in the prevalence of hematozoa between different years, seasons, or study sites. More than 60% of all recorded hemosporidian infections were light (<0.1%). The maximum level of parasitemia was recorded in the wood stork *(Mycteria americana)* and the silver-throat tanager *(Tangara icterocephala),* with 2 and 4.3% of red blood cells infected with *Haemoproteus* spp., respectively. All *Trypanosoma* infections were light, with <1 parasite per 10,000 red blood cells scanned. On the contrary, numerous microfilarial infections (37%) were heavy, with one to two embryos per 10,000 red blood cells scanned and up to three embryos per 1,000 red blood cells in the great-tailed grackle (*Quiscalus mexicanus*).

The identification of species of hemosporidians was possible when the intensity of parasitemia was >0.01% and when fully grown parasites were present on the slides. *Haemoproteus archilochus* was identified in the ruby-throated hummingbird (*Archilochus colubris*); *Haemoproteus coatneyi* in the lesser greenlet (*Hylophilus decurtatus*), western tanager (*Piranga ludoviciana*), and silver-throat tanager; *Haemoproteus plataleae* in the wood stork; *Leucocytozoon dubreuili* in the wood thrush (*Hylocichla mustelina*); *Trypanosoma avium* in the cinnamon becard (*Pachyramphus cinnamomeus*); *Trypanosoma everetti* in the great curassow (*Crax rubra*), bare-crowned antbird (*Gymnocichla nudiceps*), cinnamon becard, and barred antshrike (*Thamnophilus doliatus*). Only two *Plasmodium* spp. infections were seen in the study (Table 1); these parasites belong to the subgenera *Haemamoeba* (the host was the western tanager) and *Novyella* (the host was the lesser greenlet). Embryonic filariid nematodes cannot be identified without finding adult worms, which parasitize the body cavity and tissues of hosts.

The diversity of hematozoa recorded in the present study is typical for American birds. Furthermore, the most parasitized birds belong to families that are typically heavily infected in the Neotropics (White et al., 1978). The species of hematozoa found in the present study are common in the New World, and *L. dubreuili, T. avium,* and *T. everetti* are cosmopolitan (Bennett et al., 1982; Valkiūnas, 1997). Therefore, records of these parasites in Costa Rica are not unexpected.

Twelve species of birds (Table 1) were examined for hematozoa for the first time during the present study, according to Bennett et al. (1982), Bishop and Bennett (1992), Young et al. (1993), Valkiūnas (1997), and Valkiūnas et al. (2003). The lesser greenlet, western tanager, and silver-throat tanager are new hosts for *H. coatneyi.* All *Trypanosoma* infections identified to species were recorded in new hosts. Unidentified parasites of the genera *Haemoproteus, Plasmodium,* and microfilariae were found for the first time in seven, one, and seven bird species, respectively (Table 1).

Only four of the North American migrant birds species were infected with blood parasites (Table 1). These were the Tennessee warbler (*Vermivora peregrina*) (Parulidae), western tanager (Thraupidae), ruby-throated hummingbird (Trochilidae), and wood thrush (Turdidae). It is worth noting that blood parasites of all recorded

genera were found in resident year-round birds in the ACG. These birds can be infected only at the study site. Therefore, the transmission of representatives of all genera of hematozoa takes place in the ACG. The regularities of the transmission and vectors of the parasites remain unknown at the study site. Infection patterns in winter migrants from North America were similar to those seen in resident birds. However, the western tanager is notable, given the multiple infection of this single bird by *Haemoproteus, Plasmodium, Trypanosoma* spp., and microfilariae (Table 1). Species of the Thraupidae have been recorded to harbor these parasites in North America (Greiner et al., 1975). Therefore, some of these infections could have been acquired on the Nearctic breeding grounds. Further studies of the DNA of parasites are needed to understand the origin of the hematozoa recorded in the migratory birds, as well as the opportunity of their transmission in the ACG.

Only one study has previously reported avian blood parasites in Costa Rica. Young et al. (1993) sampled 479 birds representing 60 species in Monteverde and found an overall prevalence of infection of 11%. We found no significant difference in the overall prevalence of infection in birds sampled in Monteverde and in our study. However, *Haemoproteus* spp. were significantly more prevalent ($P<0.05$), and *Trypanosoma* spp. and microfilariae were less prevalent ($P<0.05$ and $P<0.001$, respectively) in Monteverde than in the ACG. The differences may reflect the host species sampled. The high prevalence of microfilarial infection in the present study (8%) is the highest yet recorded in birds of Central America (White et al., 1978; Young et al., 1993). It is probable that active transmission of filariid nematodes takes place in the ACG.

The low overall prevalence of avian hematozoa in our study is in accord with the results of earlier studies in Central and South America (White et al., 1978; Sousa and Herman, 1982; Woodworth-Lynas et al., 1989; Young et al., 1993; Valkiūnas, 1997; Valkiūnas et al., 2003). An intriguing regularity of the geographic distribution of avian hemosporidian parasites in birds of Central and South America is the low overall prevalence of their infections (approximately 10%) in comparison to any other zoogeographic regions (Greiner et al., 1975; White et al., 1978; Young et al., 1993; Valkiūnas, 1997). The reasons for the paucity of hemosporidian infections in the Neotropics remain unclear. It is possible that avian hemosporidians evolved in the tropics of the Old World, where they are widely distributed and prevalent (Garnham, 1966, 1980; Bennett et al., 1982; Bishop and Bennett, 1992; Valkiūnas, 1997). They probably penetrated to Central and South America through the Nearctic region of the Holarctic recently, perhaps after the last glaciation period, approximately 10,000–12,000 yr ago (Valkiūnas, 1997). Therefore, they may be less adapted and more virulent for indigenous species of birds (Valkiūnas, 1997; Valkiūnas et al., 2003). Lethal *Plasmodium* spp. infections in juvenile storklike birds and chickens in South America may be illustrations of high virulence of malaria parasites for the Neotropical birds (Garnham, 1980; Gabaldon and Ulloa, 1980; Valkiūnas, 1997).

Detailed epidemiologic and demographic studies of the parasites, vectors, and hosts supplemented by experimental work are needed to understand fully the pathogenicity of blood parasites in wild birds, as well as their distribution and significance in the Central and South America. It is important to note that prevalence data reflect the strength and duration of the infection (Valkiūnas et al., 2003). The low prevalences and intensities of hematozoa may be due, in part, to bias in the sampling of infected bird. Passerines are weakly mobile and secretive during the primary acute attack of hemosporidian parasites. They rarely enter mist nets and are not easy to collect by firearm (Valkiūnas, 2001). If birds survive the primary

acute infection, the infections become chronic, and hosts return to normal. Therefore, chronic infections, which are relatively benign, usually are found in birds captured in mist nets and collected by shooting (Valkiūnas, 2001; Valkiūnas et al., 2003). Other methods should be used to clarify the influence of blood parasitic infections on wild birds. During field studies, sampling of nestlings of species whose offspring remain in the nests for a long period of time, such as the representatives of the families Accipitridae, Columbidae, Strigidae, Coraciidae, Ciconiidae, Corvidae, and others, may help solve this question. Nestlings of these birds may be taken from the nest after 17 days of age. The great majority of hematozoan infections are patent after this time (Garnham, 1966; Baker, 1976; Valkiūnas, 1997). This method provides opportunities to record and measure heavy infections in wild birds, as well as to understand the physiologic costs of the infections to hosts and the resulting ecologic and evolutionary consequences of the diseases. Data on high prevalence (>50%) of malaria parasites in nestlings of storklike birds in Venezuela (Gabaldon and Ulloa, 1980) and hemoproteid infections in owls in Europe (Valkiūnas, 1997) are examples.

LITERATURE CITED

BAKER, J. R. 1976. Biology of the trypanosomes of birds. *In* Biology of the Kinetoplastida, Vol. 1, W. H. R. Lumsden and D. A. Evans (eds.). Academic Press, London, UK, pp. 131–174.

BENNETT, G. F., M. WHITEWAY, AND C. WOODWORTH-LYNAS. 1982. A host-parasite catalogue of the avian haematozoa. Occasional Papers in Biology, Memorial University of Newfoundland 5: 1–243.

BISHOP, M. A., AND G. F. BENNETT. 1992. Host-parasite catalogue of the avian haematozoa, Supplement 1, and Bibliography of the avian blood-inhabiting haematozoa, Supplement 2. Occasional Papers in Biology, Memorial University of Newfoundland 15: 1–244.

GABALDON, A., AND G. ULLOA. 1980. Holoendemicity of malaria: An avian model. Transactions of the Royal Society of Tropical Medicine and Hygiene 74: 501–507.

GARNHAM, P. C. C. 1966. Malaria parasites and other Haemosporidia. Blackwell Scientific Publications, Oxford, UK, 1,114 pp.

———. 1980. Malaria in its various vertebrate hosts. *In* Malaria. Epidemiology, chemotherapy, morphology, and metabolism, Vol. 1, J. P. Kreier (ed.). Academic Press, New York, New York, pp. 95–144.

GODFREY, R. D., A. M. FEDYNICH, AND D. B. RENCE. 1987. Quantification of hematozoa in blood smears. Journal of Wildlife Diseases 23: 558–565.

GREINER, E. C., G. F. BENNETT, E. M. WHITE, AND R. F. COOMBS. 1975. Distribution of the avian hematozoa of North America. Canadian Journal of Zoology 53: 1762–1787.

JANZEN, D. H. 1996. Costa Rican natural history. University of Chicago Press, Chicago, Illinois, 816 pp.

SOUSA, O. E., AND C. M. HERMAN. 1982. Blood parasites of birds from Chiriqui and Panama provinces in the Republic of Panama. Journal of Wildlife Diseases 18: 205–221.

VALKIŪNAS, G. 1997. Bird Haemosporida. Acta Zoologica Lituanica 3–5: 1–607. [In Russian with English summary].

———. 2001. Blood parasites of birds: Some obstacles in their use in ecological and evolutionary biology studies. Avian Ecology and Behaviour 7: 87–100.

———, P. SALAMAN, AND T. A. IEZHOVA. 2003. Paucity of hematozoa in Colombian birds. Journal of Wildlife Diseases 39: 445–448.

WHITE, E. M., E. C. GREINER, G. F. BENNETT, AND C. M. HERMAN. 1978. Distribution of the hematozoa of Neotropical birds. Revista de Biologia Tropical 26: 43–102.

WILKINSON, L. 1989. SYSTAT: The system for statistics. SYSTAT, Inc., Evanston, Illinois, 638 pp.

WOODWORTH-LYNAS, C. B., J. R. CAINES, AND G. F. BENNETT. 1989. Prevalence of avian haematozoa in Sao Paulo State, Brazil. Memorias do Institito Oswaldo Cruz, Rio de Janeiro 84: 515–526.

YOUNG, B. E., M. C. GARVIN, AND D. B. MCDONALD. 1993. Blood parasites in birds from Monteverde, Costa Rica. Journal of Wildlife Diseases 29: 555–560.