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HEALTH EVALUATION OF GALAPAGOS HAWKS (*BUTEO GALAPAGOENSIS*) ON SANTIAGO ISLAND, GALAPAGOS

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ABSTRACT: Galapagos Hawks (*Buteo galapagoensis*), the only endemic, diurnal raptor species in Galapagos, are currently distributed on eight Galapagos Islands having been extirpated from three of the human-inhabited islands. In January 2009, we performed health assessments of 89 Galapagos Hawks on Santiago Island, Galapagos. Four of the 89 Galapagos Hawks (4%) evaluated had physical abnormalities. Blood parameters did not differ between males and females, except for aspartate transaminase values, which were significantly higher in females than males. No Galapagos Hawks tested positive for antibodies to avian encephalitis virus, Marek virus, and paramyxovirus-1 or to haemosporidian antigen. *Chlamydophila psittaci* antigen was detected in 2 of 86 Galapagos Hawks (2%), with 24 of 43 Galapagos Hawks (56%) antibody-positive for avian adenovirus-1 and 1 of 48 Galapagos Hawks (2%) antibody positive for *Toxoplasma gondii*. There were no significant differences in infectious disease results based on sex. This study contributes to the understanding of the health status of the Galapagos Hawk and to the establishment of baseline information for the species.

Key words: Avian adenovirus-1, biochemistry profile, *Buteo galapagoensis*, *Chlamydophila psittaci*, *Toxoplasma gondii*.

INTRODUCTION

The Galapagos Hawk (*Buteo galapagoensis*) is the only endemic, diurnal raptor in Galapagos. More than one-half of the populations are on Santiago and Isabela islands (Parker, 2009). Hawks have been extirpated from three islands and are listed as threatened because of the limited population and low genetic diversity (Parker, 2009; BirdLife International, 2010). Although much is known about the life history aspects, such as diet (de Vries, 1973), social structure (Faaborg et al., 1995; DeLay et al., 1996; Bollmer et al., 2003), and genetics (Bollmer et al., 2005) of these birds, comparatively little is known about their health status. Studies on the ectoparasites, immunity, and population sizes of Galapagos Hawks show the lower innate immunity and higher ectoparasite load of hawks living on smaller islands compared with those on larger islands (Whiteman et al., 2006). We compared blood values and evidence of infection and exposure to selected infectious disease agents in male and female Galapagos Hawks on Santiago Island, Galapagos.

METHODS

Study area and field sampling

Field work was conducted at the beginning of the breeding season in January 2009 on Santiago Island, Galapagos, at Sullivan Bay (90°33′59″W, 0°17′20″S) and at James Bay (90°49′43″W, 0°12′35″S). Santiago is an uninhabited, 585-km^2 island, located in the center of the archipelago, with its highest elevation at 907 m (Jackson, 1993). Habitat types at our research sites on Santiago Island include an arid coastal zone, a transitional zone, and barren pahoehoe, and A'a lava fields (Jackson, 1993).

Six Galapagos Hawks were caught with rat-baited bal-chatri traps (Berger and Mueller, 1959), and the remainder with goat-meat–baited tangle nooses (Faaborg et al., 1980). Any Galapagos Hawk not previously banded received aluminum and anodized color bands bearing an alphanumeric code. Morphometric measurements, including wing chord and body mass, were taken as previously described, and wing chord was used to categorize

each individual as male or female because that measure does not overlap between the sexes (Bollmer et al., 2003).

A cloacal swab was collected (Fisherbrand® Sterile Swabs, Fisher Scientific, Pittsburgh, Pennsylvania, USA), individually placed in cryotubes (Nalgene NUNC International, Rochester, New York, USA), and frozen in liquid nitrogen (−196 C) in the field and mechanical freezers (−80 C) in the laboratory. Approximately 6 ml of blood (<1% of body weight) was collected from the ulnar vein. Fifty µl of blood was stored in 500 µl lysis buffer preservative solution (Longmire et al., 1988) for future molecular analyses (i.e., hemoparasite identification). The remainder of the blood was transferred to lithium heparin (Corvac, Sherwood Medical, St. Louis, Missouri, USA) and serum separator tubes (Corvac, Sherwood) and kept in a cooler until processed. All blood samples were processed between 2 and 7 hr, with 90% processed within 5 hr. Heparinized blood was used for determination of packed cell volumes (PCV) using a portable 12-volt centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA), and plasma total solids (TS) were measured using a temperature-calibrated hand-held refractometer (Schulco, Toledo, Ohio, USA). Serum separator tubes were centrifuged for 10 min and the serum was decanted, placed in cryotubes, and frozen in liquid nitrogen (−196 C) or mechanical freezers (−80 C) in the field and laboratory, respectively.

Samples were imported to the United States at room temperature (i.e., blood in Longmire buffer) or frozen (−70 C) on dry ice (i.e., swabs, sera). When applicable, samples were shipped by courier on ice to a laboratory for diagnostic testing.

Laboratory analyses

Chemistry profiles were processed on an advanced Synchron CX5 PRO Clinical System spectrophotometer (Beckman-Coulter, Inc., Bree, California, USA) by a commercial laboratory (Advanced Veterinary Laboratory, St. Louis, Missouri, USA). Hemosporidia were assayed by polymerase chain reaction (PCR) amplification of the mitochondrial cytochrome b gene (Sambrook and Russell, 1989; Waldenstrom, 2004) at the University of Missouri–St. Louis (St. Louis, Missouri, USA). Galapagos Dove (*Zenaida galapagoensis*) and Galapagos Penguin (*Spheniscus mendiculus*) blood samples infected with *Haemoproteus* sp. and *Plasmodium* sp., respectively, were used as positive controls in each test. The negative control consisted of all reagents, without DNA. Cloacal swab samples were tested for *Chlamydophila psittaci* DNA by PCR at the Infectious Diseases Laboratory at the University of Georgia College of Veterinary Medicine (Athens, Georgia, USA; Sayada et al., 1995).

Serologic tests were performed at the National Veterinary Services Laboratories (Ames, Iowa, USA) for avian adenovirus-1 by agar gel immunodiffusion (Jakowski and Wyand, 1972), and at the Veterinary Medical Diagnostic Laboratory, University of Missouri–Columbia College of Veterinary Medicine (Columbia, Missouri, USA) for avian encephalitis virus (picornavirus) by enzyme-linked immunosorbent assay (ELISA; cutoff>400; OIE, 2008), avian paramyxovirus/Newcastle disease by hemagglutination inhibition (cutoff>64; Allan and Gough, 1974), and Marek disease (herpesvirus) by agar gel immunodiffusion (OIE, 2008). The modified agglutination test (MAT) was performed using whole *Toxoplasma gondii* tachyzoites of the RH strain fixed in formalin and mercaptoethanol as described (Dubey and Desmonts, 1987) for the detection of *T. gondii*–specific antibodies. Positive and negative avian serum controls were included in each run of the MAT. A titer ≥1:50 was considered positive.

Statistical analysis

A condition index was calculated as the residual of the regression of body mass

against wing chord for both sexes combined (Green, 2001). A two-tailed *t*-test was used to compare condition index scores of Galapagos Hawks by sex (Petrie and Watson, 2006). All other numerical data were inspected for normality; two-tailed *t*-tests were performed on normal data, and Mann-Whitney *U*-tests were used where normality was rejected to compare by sex (Petrie and Watson, 2006). Prevalence was defined as the proportion of tested Galapagos Hawks with positive antibody test results, with 95% confidence intervals given (Thrusfield, 2007). Chi-squared tests or Fisher's exact tests were used to compare prevalence data by sex. Statistical significance was set as $P<0.05$. Data were analyzed using commercial statistical software (NCSS, Kaysville, Utah, USA).

RESULTS

Physical examinations of 89 Galapagos Hawks were performed and morphometrics, blood values, and evidence of infection or exposure to selected infectious agents determined for a subset of those Galapagos Hawks. As found in a previous, more extensive, across-island analysis of morphology (Bollmer et al., 2003), females ($n=31$; mean±SD=1,235±148 g; range=950–1,520 g) were heavier than males ($n=33$; mean± SD=908±89 g; range=760–1,070 g; *t*-test, $P<0.0001$). Although females were larger, there was no significant difference (*t*-test, $P=0.17$) in condition index scores between sexes.

Two of the 48 males (4%) and two of 41 females (5%) had physical abnormalities. One male was subjectively rated as extremely thin (i.e., pronounced keel bone), and another was thin with poor feathers (i.e., frayed with stress bars) and a grade III/VI heart murmur. The two females with abnormalities included one individual with poor feathers and a second that had a heavy ectoparasite infestation consisting of louse flies (*Icosta nigra*) and lice (*Colpocephalum turbinatum* and *Degeeriella regalis*). These four individuals were excluded from morphometrics and blood parameter data sets but included in data for evidence of infection or exposure to infectious disease-causing agents. Male and female Galapagos Hawks had similar PVC, TS, and biochemistry values for all parameters, except AST, which was significantly higher in females (Table 1; Mann-Whitney *U*-test, $P=0.048$).

There was no evidence of infection with blood parasites ($n=76$) or exposure to avian encephalitis virus ($n=43$), Marek disease virus ($n=43$), or paramyxovirus-1 ($n=43$). Two of 86 Galapagos Hawks (1 female, 1 male) were positive for *C. psittaci* (prevalence=2%; 95% CI=0.3–8.5%), 1 of 48 Galapagos Hawks was positive to *T. gondii* (1:800 titer, prevalence=2%; 95% CI=0.1–11.5%), and 24 of 43 Galapagos Hawks (11 females, 13 males) had antibodies to avian adenovirus-1 (prevalence=56%; 95% CI=39.9–70.9%).

DISCUSSION

This is the first study, to our knowledge, to obtain PCV, TS, biochemistry values, and infectious disease-causing agent data for Galapagos Hawks. Some limitations exist with the blood values because blood processing was delayed up to 7 hr for a few Galapagos Hawks, and white blood cell differentials were not available because of a logistic error that rendered blood smears unusable.

The significantly higher AST value in females may be due to increased muscle trauma in the larger birds because CK, as an indicator of muscle damage, was also higher, although not statistically significant, in females (Fudge, 2000b). The mean calcium values for both males and females were low compared with a number of captive raptor species (ISIS, 2002); however, they were similar to a study in free-living Northern Goshawks (*Accipiter gentilis*) that found a mean of 4.7 mg/dl (1.18 mmol/l) in females (Hanauska-Brown

TABLE 1. Blood values for Galapagos Hawks (*Buteo galapagoensis*) on Santiago Island, Galapagos.

Parameter	All hawks	Males	Females
Packed cell volume (%)			
$\bar{x}$ (SD)	43 (3.1)	43 (3.6)	44 (3.0)
Median	44	44	44
Range	34–50	34–50	38–49
n	63	35	28
Total solids (g/l)			
$\bar{x}$ (SD)	41.9[a] (5.5)	41.1[a] (6.1)	42.9[a] (4.6)
Median	42	40	43
Range	20–56	20–56	31–55
n	63	35	28
Total protein (g/l)			
$\bar{x}$ (SD)	33 (5.2)	32 (5.0)	35 (5.2)
Median	32.5	32	35
Range	21–45	21–43	27–45
n	48	27	21
Albumin (g/l)			
$\bar{x}$ (SD)	12.5[a] (1.4)	12.3[a] (1.2)	12.8[a] (1.7)
Median	12	12	13
Range	10–16	10–14	10–16
n	48	27	21
Globulin (g/l)			
$\bar{x}$ (SD)	21 (4.6)	20 (4.5)	22 (4.6)
Median	21	19	23
Range	11–31	11–31	14–31
n	48	27	21
Sodium (mmol/l)			
$\bar{x}$ (SD)	152[a] (8.4)	152[a] (9)	154[a] (7)
Median	154	153	155
Range	123–170	123–170	135–165
n	48	27	21
Potassium (mmol/l)			
$\bar{x}$ (SD)	2.0[a] (0.6)	2.1[a] (0.6)	2.0[a] (1)
Median	1.9	2	1.7
Range	1.5–4.6	1.5–4.6	2–3
n	48	27	21
Chloride (mmol/l)			
$\bar{x}$ (SD)	121 (6.9)	120 (7)	122 (6)
Median	121	120	124
Range	101–134	101–134	109–133
n	48	27	21
Calcium (mmol/l)			
$\bar{x}$ (SD)	1.97 (0.27)	1.92 (0.25)	2.02 (0.30)
Median	1.95	1.93	1.98
Range	1.35–2.45	1.38–2.45	1.35–2.35
n	48	27	21
Phosphorus (mmol/l)			
$\bar{x}$ (SD)	0.80[a] (0.34)	0.84[a] (0.39)	0.76 (0.28)
Median	0.7	0.68	0.74
Range	0.36–2.23	0.42–2.23	0.36–1.42
n	48	27	21

TABLE 1. Continued.

Parameter	All hawks	Males	Females
Glucose (mmol/l)			
$\bar{x}$ (SD)	15.11 (2.09)	14.79 (2.04)	15.52 (2.11)
Median	15.35	14.99	15.71
Range	10.49–18.76	10.49–18.09	10.82–18.76
n	48	27	21
AST (u/l)[b]			
$\bar{x}$ (SD)	194*[a] (83)	178[a] (95)	216 (61)
Median	176	153	208
Range	94–517	94–517	125–331
n	48	27	21
Total bilirubin (μmol/l)			
$\bar{x}$ (SD)	8.2[a] (9.0)	8.0[a] (11.1)	8.5[a] (5.4)
Median	5.13	5.13	8.6
Range	0–56.4	0–56.4	1.7–23.9
n	48	27	21
Uric acid (μmol/l)			
$\bar{x}$ (SD)	1,101[a] (1,908)	899[a] (521)	1,360[a] (2,842)
Median	655	797	583
Range	37–13,621	36–2,272	297–13,621
n	48	27	21
Creatinine kinase (u/l)			
$\bar{x}$ (SD)	311[a] (261)	284[a] (179)	345[a] (340)
Median	231	235	220
Range	34–1,285	109–826	34–1,285
n	48	27	21

[a] Assumption of normality was not met for this parameter.

[b] AST = aspartate aminotransferase.

* Male and female values were significantly different ($P<0.05$).

et al., 2003). In a study of 33 free-living, nestling Montagu's Harriers (*Circus pygargus*) calcium values ranged from 1.07–2.49 mmol/l (Limiñana et al., 2009). The uric acid values for the Galapagos Hawks were high compared with other free-living raptor species and may be elevated because of postprandial sampling of many of the Galapagos Hawks, which had eaten just before blood collection because some Galapagos Hawks were baited with food (Stein et al., 1998; Fudge, 2000a; Hanauska-Brown et al., 2003; Sarasola et al., 2004).

Evidence of exposure to all the infectious agents we tested for has been detected in other avian species in Galapagos or in other raptor species (Deem, 1999; Padilla et al., 2003, 2004; Gottdenker et al., 2005; Travis et al., 2006a, b; Soos et al., 2008; Deem et al., 2010). The high avian adenovirus-1 antibody prevalence in the study population is similar to that determined in backyard chickens (*Gallus gallus*) and Flightless Cormorants (*Phalacrocorax harrisi*) in the Galapagos (Travis et al., 2006a; Soos et al., 2008). These studies suggest that adenoviruses are circulating in many species of birds in Galapagos or that the test is uniformly nonspecific and, therefore, gives similar prevalence data, regardless of species. Although Santiago Island is free of domestic cats (*Felis catus*), the definitive host of *T. gondii*, one of the 48 Galapagos Hawks tested had antibodies against *T. gondii*. An earlier study in which 2.3% of Galapagos Penguins and Flightless Cormorants were positive for *T. gondii*

antibodies on islands with and without cats also demonstrated exposure in free-living Galapagos birds (Deem et al., 2010). Galapagos Hawks on Santiago Island may be exposed to *T. gondii* through consumption of bradyzoites in intermediate hosts (e.g., mammals), oocysts in cat feces arriving from surrounding islands that have cats, or spillover from consumption of domestic chickens. For example, the antibody-positive Galapagos Hawk was sampled in James Bay, a site frequented by tourists who may leave chicken scraps. Lastly, we cannot rule out a false-positive result on the MAT because this test has not been validated in Galapagos Hawks.

Chlamydophila psittaci sequences were identified in cloacal swabs from two Galapagos Hawks. Because of the intermittent shedding of this organism, our results may underestimate the true prevalence of this pathogen. Other studies in Galapagos have shown positive results in Rock Doves (*Columba livia*; Padilla et al., 2004), Flightless Cormorants, and Galapagos Penguins (Travis et al., 2006a, b), and domestic chickens (Gottdenker et al., 2005; Soos et al., 2008). Endemic worldwide, *C. psittaci* is well adapted to avian hosts and has been confirmed as a pathogen in other raptor species (Fowler et al., 1990). Infections are often asymptomatic, but severity depends on the virulence of the strain and host species susceptibility (Gerlach, 1994).

None of the Galapagos Hawks tested in this study had detectable antibodies to avian encephalitis virus, Marek disease virus, or paramyxovirus-1, all three of which have been identified in wild and domestic birds in Galapagos (Padilla et al., 2003; Gottdenker et al., 2005; Soos et al., 2008). To detect antibody with 95% confidence of finding at least one positive and based on 10% prevalence, we needed to test 28 Galapagos Hawks if using a diagnostic test that has 100% sensitivity and specificity (Cannon and Roe, 1982). Although the sensitivity and specificity of these tests are unknown for the Galapagos Hawk, 43 Galapagos Hawks were tested. Findings from this study will be important for future comparisons to the Galapagos Hawk populations on Santiago Island and other Galapagos Hawk populations in the archipelago from islands with and without human inhabitants.

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