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HEMATOLOGY, PLASMA CHEMISTRY, SEROLOGY, AND *CHLAMYDOPHILA* STATUS OF THE WAVED ALBATROSS (*PHOEBASTRIA IRRORATA*) ON THE GALAPAGOS ISLANDS

Luis R. Padilla, D.V.M., Kathryn P. Huyvaert, M.S., Jane Merkel, L.V.T., R. Eric Miller, D.V.M., Dipl. A.C.Z.M., and Patricia G. Parker, Ph.D.

Abstract: Venipuncture was performed on 50 adult, free-ranging waved albatrosses (*Phoebastria irrorata*) on Española, Galapagos Islands, Ecuador, to establish hematologic and plasma biochemistry reference ranges and to determine the prevalence of exposure to important domestic avian pathogens. Weights and plasma creatine phosphokinase activities differed significantly between males and females. Serum was tested for evidence of exposure to avian influenza, avian paramyxoviruses 1, 2, and 3, avian cholera, adenovirus groups 1 and 2, avian encephalomyelitis, Marek's disease, infectious bursal disease, and infectious bronchitis virus (Connecticut and Massachusetts strains). Of 44 birds, 29 (66%) seroreacted to adenovirus group 1, and four seroreacted to avian encephalomyelitis. Cloacal swabs were negative for *Chlamydophila psittaci* DNA.

Key words: Waved albatross, *Phoebastria irrorata*, hematology, plasma chemistry, serology, *Chlamydophila psittaci*.

INTRODUCTION

The colonial waved albatross (*Phoebastria irrorata*), a member of the Procellariformes, breeds in only two places: on the islands of Española in the Galapagos Islands of Ecuador[1,9] and in La Plata near the west coast of Ecuador. Less than 20 breeding pairs have been reported on La Plata,[1] and recent surveys suggest that the size of the Española population, which contains 15,000–17,000 pairs,[1] has been stable for 25 yr. Using criteria set by the International Union for Conservation of Nature and Natural Resources, the waved albatross could be considered ''vulnerable'' on the basis of its restricted breeding range.[3] As with other seabird species,[20, 21] colonial breeding habits and limited geographic distribution could make this species susceptible to epizootics and to natural or anthropogenic environmental disasters. The long distances traveled by waved albatrosses to unique foraging grounds along the South American coast may expose them to environmental factors and infectious diseases not encountered by birds that never leave the archipelago. The natural history, ecology, conservation, and management of this species,[1,9] but not its health status, have been described.

Baseline health parameters serve as reference points for future population assessments and are essential for determining population health.[10,18] This study sought baseline hematologic and plasma chemical data for the waved albatross and data on the prevalence of exposure to important domestic avian pathogens so that epizootics and other health-related threats to the population could be recognized and managed. Captive procellarids are uncommon, so reference ranges were established from wild populations.

MATERIALS AND METHODS

Fifty adult, clinically healthy waved albatrosses (22 males, 28 females) were physically restrained during July, 2001, on Punta Cevallos, Island of Española, Galapagos Islands, Ecuador (1°S, 98°W), for ulnar vein venipuncture, cloacal swab collection, and weight determination. Techniques had been approved by the University of Missouri–Saint Louis Institutional Animal Care and Use Committee. Sex was determined by morphology and behavior and confirmed by a polymerase chain reaction (PCR) molecular technique.[4]

Whole-blood samples (1–8 ml) were collected from 50 birds. Blood smears were made from 45 samples using fresh, unpreserved blood. These were fixed and stained with a modified Wright–Giemsa stain (JorVet Dip-Quick, Jorgensen Laboratories, Loveland, Colorado 80538, USA) for hemoparasite examination. Blood was placed in lithium heparin tubes or serum collection tubes with separator gel and clot activator (Vacutainer System, Becton-Dickinson, Franklin Lakes, New Jersey 07417, USA). Serum and plasma were harvested after in-field centrifugation for 20–30 min using a battery-powered centrifuge unit (Mobilespin Model 128, Vulcon Technologies, Grandview, Missouri

From the Saint Louis Zoological Park, 1 Government Drive, St. Louis, Missouri 63110, USA (Padilla, Merkel, Miller, Parker); College of Veterinary Medicine, University of Missouri–Columbia, 203 Veterinary Medicine Building, Columbia, Missouri 65211, USA (Padilla, Miller); and Department of Biology, University of Missouri–Saint Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA (Huyvaert, Parker). Correspondence should be directed to Dr. Padilla.

Table 1. Weight and estimated hematology parameters for free-ranging adult waved albatrosses.

Parameter	Mean	Range	*N*
Weight (kg)	3.7 ± 0.6	2.8–5.0	47
Packed cell volume (L/L [%])	0.4 ± 0.05 (38.2 ± 5.1)	0.24–0.50 (24–50)	28
Estimated leukocyte count ($\times 10^9$/L)	5.9 ± 2.4	1.5–11.1	45
Heterophils (%)	66.1 ± 30	1.1–8.9	45
Monocytes (%)	1.7 ± 1.7	0–0.5	45
Lymphocytes (%)	30.5 ± 15	0.27–3.9	45
Eosinophils (%)	1.7 ± 1.7	0–0.4	45
Basophils (%)	0.0 ± 0.01	0–0.2	45

64030, USA) at 3,000 rpm. After separation, plasma and serum were transferred to cryotubes (Nalge Nunc International, Rochester, New York 14625, USA) and frozen at ≤−20°C. All samples were frozen in liquid nitrogen for preservation and transport until laboratory analysis. Fresh blood samples from 28 of the birds (12 males, 16 females) were placed in heparinized microcapillary tubes, and packed cell volumes (PCVs) were measured in the field after centrifugation. Fixed blood smears and plasma were submitted to a commercial veterinary laboratory (Antech Diagnostics, Chicago, Alsip, Illinois 60903, USA) for estimated total and differential white blood cell (WBC) counts, detection of hemoparasites, and automated plasma chemistry profiles. The estimated WBC count is the standard protocol for this commercial laboratory.[6,11]

Cloacal swab samples were transferred to cryotubes, frozen in liquid nitrogen, and submitted for detection of *Chlamydophila psittaci* DNA sequences by PCR to the Infectious Diseases Laboratory, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA.

Serum samples from 44 birds (20 males, 24 females) were tested for antibodies to adenovirus group 1, avian encephalomyelitis virus, Marek's disease, and infectious bursal disease by agar gel immunodiffusion (AGID); for antibodies to infectious bronchitis virus (Connecticut and Massachusetts strains) and avian paramyxovirus 2 and 3 by hemagglutination inhibition; and for antibodies to reovirus by immunofluorescence at the National Veterinary Services Laboratory, Ames, Iowa 50010, USA. Samples were also tested for antibodies to adenovirus group 2 (hemorrhagic enteritis of turkeys) and avian influenza by AGID and for avian paramyxovirus 1 (Newcastle's disease) and avian cholera by microagglutination at the Diagnostic Laboratory of the University of Missouri–Columbia, College of Veterinary Medicine, Columbia, Missouri 65211, USA. Insufficient serum volumes precluded serologic testing of the other six birds.

Plasma samples with gross evidence of hemolysis and individual test results that were more than 3.0 standard deviations from the mean were excluded from analysis. Although ideal reference ranges are calculated using 1.96 standard deviations from the mean,[8] the limited sample size was taken into account for establishing these reference ranges. A commercial statistical software package (NCSS®, Number Cruncher Statistical Systems, Kaysville, Utah 84037, USA) was used for data analysis. Mean and standard deviation were calculated for weights, PCV, estimated WBC count, and plasma chemistry values. Results were visually inspected for normal distribution, and normality was confirmed by a Shapiro–Wilk *W* test for all chemistry, PCV, weight, and estimated total WBC count values. To explore potential sex-related biases, the data set was further subdivided by sex, and a two-sample *t*-test was used to identify differences between males and females. Similarly, plasma chemistries and estimated WBC counts were compared between seroreactive and seronegative birds for all the diseases tested. Because the means of 16 parameters were compared for each subset of the data, a Bonferroni-corrected *P*-value ≤0.003 (0.05/16) was considered significant.[14]

RESULTS

Weights, leukograms, and plasma chemistry values are shown in Tables 1, 2. No hemoparasites were identified in the blood smears examined ($n = 45$). Males weighed significantly more than females ($P < 0.003$; 4.1 ± 0.5 kg, $n = 20$ and 3.3 ± 0.4 kg, $n = 27$, respectively). Creatine phosphokinase (CPK) activity was significantly lower ($P < 0.003$) in males (196.4 ±109.4 U/L, $n = 19$) than in females (370.9 ± 178.9 U/L, $n = 22$). After Bonferroni correction, mean values of aspartate aminotransferase (AST) for females and males (133.6 ± 47.1 U/L, $n = 25$ and 99.4 ± 40.3 U/L, $n = 22$, respectively) and of cholesterol for females and males (7.0 ± 1.3 mmol/L, $n = 26$ and 6.0 ± 1.3

Table 2. Plasma chemistry parameters for free-ranging adult waved albatrosses.

Parameter	Mean	Range	*N*
Total protein (g/L [g/dL])	45.0 ± 6.0 (4.5 ± 0.6)	35–57 (3.5–5.7)	48
Albumin (g/L [g/dL])	18.0 ± 2.0 (1.8 ± 0.2)	15–22 (1.5–2.2)	48
Globulin (g/L [g/dL])	28.0 ± 5.0 (2.8 ± 0.5)	19.0–38.0 (1.9–3.8)	47
P (mmol/L [mg/dL])	1.1 ± 0.3 (3.4 ± 0.8)	0.6–1.9 (1.8–6.0)	48
Ca (mmol/L [mg/dL])	2.5 ± 0.3 (9.8 ± 1.1)	1.3–3.1 (5.1–12.3)	48
Glucose (mmol/L [mg/dL])	12.7 ± 2.0 (229.4 ± 35.4)	9.8–19.2 (176–346)	47
Na (mmol/L [mEq/L])	152.7 ± 6.2 (152.7 ± 6.2)	134–166 (134–166)	45
K (mmol/L [mEq/L])	3.7 ± 0.8 (3.7 ± 0.8)	2.0–5.4 (2.0–5.4)	48
Cl (mmol/L [mEq/L])	118.0 ± 7.7 (118.0 ± 7.7)	105–138 (105–138)	46
Uric acid (mmol/L [mg/dL])	0.3 ± 0.2 (4.4 ± 2.7)	0.1–0.7 (1.4–11.7)	47
Cholesterol (mmol/L [mg/dL])	6.5 ± 1.4 (251.8 ± 54.0)	3.4–10.4 (133–401)	48
Aspartate aminotransferase (U/L)	117.6 ± 46.9	45–243	47
Creatine phosphokinase (U/L)	290.0 ± 173.1	48–711	41

mmol/L, $n = 26$, respectively) were not significantly different ($P > 0.003$).

Serology results and testing techniques are presented in Table 3. Twenty-nine (66%) birds showed seroreactivity to avian adenovirus group 1 by AGID, and four (9%; three females, one male) were seroreactive to avian encephalomyelitis virus by AGID. No statistically significant differences in plasma chemistry values or estimated total WBCs were found between seropositive and seronegative individuals. All 50 cloacal swabs were negative for the presence of *Chlamydophila* DNA sequences by PCR.

DISCUSSION

These are the first published hematology and plasma chemistry reference ranges for the waved albatross. Although leukocyte counts were estimated from fixed blood smears, results of this technique may be more variable than those of hemacytometer-based techniques when performed under ideal conditions.[17] However, hemacytometer-based techniques are impractical in remote field situations, such as the island of Española, where limited access precludes the availability of quality microscopy equipment and whole-blood preservation. In these field situations, where the decline in condition of cell quality in unpreserved whole blood may create artifacts, estimated total WBC counts from freshly made and fixed blood smears are likely to provide more reliable results.[6] Fixed blood smears also provide permanent archival information,[6] which can be a useful tool for conservation and population medicine.

Table 3. Serologic test results and methodology used for free-ranging adult waved albatrosses in the Galapagos Islands ($n = 44$).

Test	Seroreactive	% seroreactivity	Method	Positive[a]
Avian adenovirus 1	29	66	AGID[b]	
Avian encephalomyelitis	4	9	AGID	
Avian cholera	0	0	MA[c]	≥1:8
Avian paramyxovirus 1	0	0	HI[d]	≥1:8
Avian paramyxovirus 2	0	0	HI	≥1:8
Avian paramyxovirus 3	0	0	HI	≥1:8
Hemorrhagic enteritis	0	0	AGID	
Marek's disease	0	0	AGID	
Avian influenza	0	0	AGID	
Reovirus	0	0	IFA	≥1:20
Infectious bursal disease	0	0	AGID	
Infectious bronchitis[e]	0	0	HI	≥1:8

[a] Titer considered positive by testing laboratory. Not applicable for AGID methodology.
[b] AGID = agar gel immunodiffusion.
[c] MA = microagglutination.
[d] HI = hemagglutination inhibition.
[e] Connecticut and Massachusetts strains.

The hematology and chemistry parameters for the waved albatross (*P. irrorata*) are generally consistent with the values for the Laysan albatross (*P. immutabilis*),[21] with minor exceptions. Although different count techniques were used, the Laysan albatrosses from three different islands[21] had higher total-WBC ranges (9.8–42.55 $\times$ 10^9/L) than those estimated for the waved albatross of the Galapagos islands (1.5–11.1 $\times$ 10^9/L). Although this may reflect species differences, inherent variability in the WBC estimation techniques[17] and sampling differences may have influenced the values. Despite differences in total WBC numbers, similar trends are observed in heterophil to lymphocyte ratios between the two species. Laysan albatross heterophil–lymphocyte ratios were roughly 1.9, 2.0, and 2.7 for the three island populations, and the waved albatross had a 2.2 ratio. In other species, these ratios vary widely. Higher heterophil–lymphocyte ratios have been reported for white storks (*Ciconia ciconia*), white pelicans (*Pelecanus erythrorhynchos*), and great frigatebirds (*Fregata minor*).[22] The significance of these variations is unknown. They may reflect species-specific idiosyncrasies or different capture, restraint, and blood collection methodologies, and they highlight the need for species-specific reference ranges and the danger of extrapolating data between species. Plasma glucose values were higher for the waved albatross than those reported for the Laysan albatross.[21] Plasma electrolytes (Na, Cl, and K) have not been reported for the Laysan albatross and, therefore, could not be compared, but all other plasma chemistry values were comparable between the two species.

The only plasma chemistry value from *P. irrorata* that differed significantly between males and females was CPK activity. Despite lack of statistical significance, sex differences in cholesterol and AST values may reflect true biological differences. In the absence of clinical signs, differences in these inherently variable enzymes likely reflect normal reproductive or physiologic processes. The possibility of an age-dependent bias in the female population cannot be ruled out because exact ages were not available for the adult individuals sampled. Because elevations in CPK are specific to muscle damage, concurrent elevations in AST are unlikely to be of hepatocellular origin.[7] Variations in plasma cholesterol between captive and recently caught brown pelicans have been attributed to dietary differences,[22] and high cholesterol levels have been associated with carnivorous birds.[5] Sex-dependent differences in foraging strategies may explain the differences in plasma cholesterol. Alternatively, elevations in plasma cholesterol have been associated with active folliculogenesis and other reproductive processes,[12] but this is an unlikely explanation for cholesterol elevations in female waved albatrosses because all birds were sampled during either late incubation or early brooding of chicks and after the active egg-laying season of April to early June.

The absence of hemoparasites in *P. irrorata* is notable because hippoboscid flies, which can be vectors of protozoal hemoparasites,[2] were observed on some of the birds. Monitoring for the presence of hemoparasites during different times of the year has intrinsic ecologic interest and might influence population management strategies.

Avian serologic responses must be interpreted cautiously because most of these tests have been validated for only domestic chickens. Test methodology, sensitivity, and specificity must be taken into account before making conclusions or generalizations about the health of a population. Unlike a study on wild rockhopper penguins (*Eudyptes chrysocome*),[10] plasma chemistry values did not differ between seroreactive and seronegative albatrosses.

Group 1 adenoviruses include 12 serologically distinct viruses that generally affect young birds, and although morbidity and mortality vary with host and virus, clinical signs are associated with hepatitis, enteritis, and respiratory disease.[16] The high prevalence (66%) of seroreactivity to group 1, with no seroreactivity to group 2 adenoviruses in our study population, suggests exposure to viruses of the first group, although none showed evidence of disease. Because the persistence or half-life of adenovirus 1–specific antibodies in albatrosses has not been studied, the relevance of seroreactivity in asymptomatic birds is unknown. Group 1 adenoviruses can be recovered from asymptomatic domestic birds, and antibody surveys suggest that most adult birds have been exposed to numerous strains.[16] Furthermore, the implications of high prevalence of seroreactivity are limited by the serology methods used. AGID serology tests have the inherent limitations of limited sensitivity and an inability to quantify antibody concentrations.[15] Because these tests rely on precipitation of unknown antigen with patient serum into antigen–antibody complexes, the possibility of cross-reaction with nonspecific proteins cannot be ruled out. Surveillance of hatchlings and juveniles for causes of morbidity and mortality may elucidate the significance of adenoviruses in the waved albatross population.

Seroreactivity to avian encephalomyelitis by AGID must also be interpreted with caution, as rationalized for group 1 adenoviruses. No birds showed clinical signs consistent with this entero-

virus, and seroreactivity may indicate evidence of previous exposure to this or other related viruses. Because undocumented enteroviruses are suspected to be associated with disease in companion and aviary birds,[13] it is possible that unidentified enteroviruses are present in the waved albatross population and cross-react to the serology methods used in this study.

Without evidence of the clinical signs of *Chlamydophila* infections, we attempted to identify subclinical, latent carriers. The PCR technique used in this study has the advantage of both high sensitivity and high specificity.[19] At the time of sampling, none of the 50 albatrosses had evidence of fecal shedding of *Chlamydophila* spp. Although a combination of serology, hematology, and cloacal and choanal DNA detection by PCR maximizes the probability of detecting true positives,[19] the absence of this organism in all cloacal swab samples suggests that *Chlamydophila* spp. either occurs at an extremely low prevalence and was not detected by our sampling methodology or is not present in the waved albatross population.

Continued surveillance for causes of morbidity and mortality and further resolution into age, sex, and seasonal and geographic differences is warranted.

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