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## Hematology and Plasma Biochemistry of Captive Puna Ibis (*Plegadis ridgewayi*)

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**ABSTRACT:** Normal hematology and plasma biochemistry values are reported for a single captive population of 14 puna ibises (*Plegadis ridgewayi*). The natural biology and conservation status of this species is virtually unknown and it is considered a vulnerable species in parts of South America. The values presented here may be useful to clinicians or wildlife managers working with puna ibises.

**Key words:** Blood values, hematology, plasma biochemistry, *Plegadis ridgewayi*, puna ibis.

The puna ibis (*Plegadis ridgewayi*) is a medium-sized (56- to 61-cm) bird indigenous to the Andean Mountains of western South America. Puna ibises are dark purplish brown in color with a dark-red, downward-curved bill. These ibises frequent large marshes and damp pasturelands and nest in tall reeds in highland marshes. Little is known about their biology or conservation status. The natural marshy vegetation of the puna zone of the Andes is threatened by human overpopulation, overharvesting of vegetation, and over-grazing by cattle. The puna ibis also is threatened by overharvesting of eggs. The puna ibis is considered a vulnerable species in South America, although some populations have recovered (Hancock et al., 1992).

Fewer than 150 puna ibises are captive throughout the world; fewer than one quarter of these are held in the USA (International Species Information System [ISIS], 2003). Of the puna ibises in captivity, only a few standard hematologic and biochemical values for one to four birds have been reported to the ISIS Physiological Data Reference Values (ISIS, 2002). This manuscript reports normal hematology and plasma biochemistry values for captive puna ibises that may be useful as

reference values for clinicians and wildlife managers.

All birds are housed outdoors in an off-exhibit holding facility at the Oklahoma City Zoological Park (Oklahoma City, Oklahoma, USA; 35°31'N, 97°28'W). They are fed a diet consisting of fresh meat (Classic Birds of Prey Diet, Nebraska Brand, North Platte, Nebraska, USA), fresh fish (smelt or capelin), krill, and a variety of pelleted diets (Flamingo-Fair, Reliable Protein Products, Palm Desert, California, USA; game bird and game fish chow, Purina Mills, St. Louis, Missouri, USA), and Wayne's dog food (Royal Canin, Inc., St. Peters, Missouri, USA). Six male and eight female puna ibises included in this study weighed between 448 and 729 g, and ranged in age from 4.5 to 22.8 yr.

During January 2002, the ibises were caught in nets and manually restrained for identification, body weight measurement, physical examination, and blood collection during a span of a couple hours in the late morning. All birds appeared to be clinically healthy upon physical examination and were released back into the enclosure.

Blood (2 ml) was collected from the right jugular vein with a 25-ga needle attached to a 3-ml syringe. The blood sample was placed into lithium heparin blood tubes (Microtainer®, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). A portion (1.5 ml) from each bird was centrifuged for 10 min, and the plasma was removed and placed into a cryovial (Corning Company, Corning, New York, USA). Samples were refrigerated and transported to the Special Medicine Laboratory at the College of Veterinary Medicine, Oklahoma State University (Stillwater, Oklahoma).

TABLE 1. Mean, standard deviation, and range of hematologic values for 14 captive puna ibises (*Plegadis ridgewayi*).

Hematology <sup>a</sup>	Mean	±SD	Range	n
WBC (×10/mm)	3.9	1.8	1.4–7.6	12
RBC (×10/mm)	3	0.3	2.54–3.68	14
Hgb (g/dl)	16.4	2.2	13.2–21.4	11
Hct (%)	42	4	36–51	14
MCV	140	3	136–145	14
Differential				
Heterophils (%)	21	10	7–42	14
Lymphocytes (%)	62	14	40–86	13
Monocytes (%)	7	4	2–15	14
Eosinophils (%)	1	1	0–3	14
Basophils (%)	12	7	1–24	14
Absolute values (×10 <sup>3</sup> /μl)				
Heterophils	1.1	0.8	0.1–2.2	14
Lymphocytes	2.0	0.9	0.7–2.5	12
Monocytes	0.3	0.2	0.1–0.6	14
Eosinophils	0.0	0.0	0.0–0.1	13
Basophils	0.5	0.4	0.1–1.1	14

<sup>a</sup> WBC = white blood cells; RBC = red blood cells; Hgb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume.

The hematocrit, red blood cell count, mean corpuscular volume, and hemoglobin (Hgb) concentration were determined by using an automated process (Cell-Dyn<sup>®</sup> 3500 System, Abbott Diagnostics, Abbott Park, Illinois, USA). Total white blood cell (WBC) count was determined by using the eosinophil unopette system (Unopette, Becton Dickinson). Blood smears from each bird were made and stained with modified Wright-Giemsa stain (Accustain<sup>™</sup>, Sigma Diagnostics, St. Louis, Missouri, USA). Each smear was evaluated for hemoparasites and hemopathologic changes. Differential counts were determined by examining 100 leukocytes and categorizing them as heterophils, lymphocytes, monocytes, eosinophils, and basophils based upon staining characteristics and morphology (Campbell, 1995).

The plasma biochemistry profile values were analyzed with a Vitros 250 Chemistry System (Ortho-Clinical Diagnostics, Johnson & Johnson Company, Rochester, New York, USA). Biochemistry values reported are sodium, potassium, chloride, blood urea nitrogen (BUN), phosphorus, glu-

cose, calcium, total protein, albumin, globulin, total bilirubin, creatine kinase (CK),  $\gamma$ -glutamyltransferase, aspartate aminotransferase (AST), alanine aminotransferase, uric acid, cholesterol, and osmolality.

Mean, standard deviation, variance, and Student's *t*-test were calculated by using standard statistical software (Microsoft<sup>®</sup> Excel v.2002, Microsoft<sup>®</sup> Corporation, Redmond, Washington, USA). Windsor test for suspected extreme values ( $P < 0.05$ ) was performed and these were excluded from the data analyses (Dixon and Massey, 1983).

Hematologic and the plasma biochemical data are presented in Tables 1 and 2. No hemoparasites or hemopathologic change was detected on each of the individual blood smears. Extreme hematologic values ( $P < 0.05$ ) were found for WBC count, Hgb, total protein, lymphocyte differential, absolute lymphocyte count, and absolute eosinophil count. Extreme plasma biochemical values ( $P < 0.05$ ) were found for CK and uric acid.

The data between known sexes (male,  $n=6$ ; female,  $n=6$ ) were analyzed based on

TABLE 2. Mean, standard deviation, and range of plasma biochemistry values for 14 captive puna ibises (*Plegadis ridgewayi*).

Plasma biochemistry <sup>a</sup>	Mean	±SD	Range	n
Sodium (mmol/l)	143	2.1	140–145	14
Potassium (mmol/l)	3.5	0.4	3–4.6	14
Chloride (mmol/l)	115	1.3	112–117	14
BUN (mg/dl)	2.1	0.4	2–3	14
Phosphorus (mg/dl)	3	1.4	1.0–4.9	14
Glucose (mg/dl)	303	57	245–438	14
Calcium (mg/dl)	9.3	0.5	8.0–9.8	14
Total protein (g/dl)	3.9	0.4	2.6–4.4	14
Albumin (g/dl)	1.5	0.2	1.0–1.7	14
Globulin (g/dl)	2.4	0.3	1.6–2.8	14
A/G ratio	0.61	0	0.53–0.69	14
Total bilirubin (mg/dl)	0.1	0.1	0.1–0.3	14
CK (U/l)	354	67	234–499	12
GGT (U/l)	11	0.8	10–12	14
AST (U/l)	176	39	117–240	14
ALT (U/l)	38	24.9	3.0–77	14
Uric acid (mg/dl)	7.59	2.6	4.03–12.15	13
Cholesterol (mg/dl)	150	20.6	113–187	14
Osmolality (mosm/kg)	284	4.5	276–290	14

<sup>a</sup> BUN = blood urea nitrogen; A/G = albumin/globulin ratio; CK = creatine kinase; GGT =  $\gamma$ -glutamyltransferase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

a two-tailed Student's *t*-test with equal or unequal variances as was appropriate for all hematologic and biochemical parameters. The only significant differences ( $P < 0.05$ ) determined were found for absolute lymphocyte count and total protein. Males in this population had an increased absolute lymphocyte count ( $2.7 \pm 0.8 \times 10^3/\mu\text{l}$ ) and total protein ( $4.2 \pm 0.2$  g/dl) as compared to the females' absolute lymphocyte count ( $1.5 \pm 0.7 \times 10^3/\mu\text{l}$ ) and total protein ( $3.9 \pm 0.2$  g/dl).

The puna ibis is a species not commonly kept in captivity and a sizable collection of animals of varying ages and nearly equal sex ratio in one facility provided an opportunity to obtain sufficient samples to provide meaningful reference ranges for the general population. The hematologic parameters were comparable to similar avian species and to the few (one to four) puna ibises listed in ISIS Physiological Data Reference Values for captive wildlife (ISIS, 2002). White blood cell counts may be affected by length of capture attempt, method of capture, disease, age, hormone

levels, diet, etc. (Maxwell, 1993; Fudge and Joseph, 2000). The relative differential revealed predominance of lymphocytes, which is common in some avian species. The study population had a marked relative increase in basophils ( $12 \pm 7\%$ ). Increases in basophils some birds may indicate acute inflammation, anaphylactic reactions, or severe stress (Maxwell and Robertson, 1995; Fudge and Joseph, 2000). The birds in this collection had been in the same outdoor enclosure for more than 5 yr. Climactic conditions may have contributed to stress on the birds because the temperature was above 27 C the day before blood collection and near 17 C the day of collection.

Plasma biochemistry profiles also revealed values comparable to other avian species and puna ibis (ISIS, 2002). Blood urea nitrogen was  $2.1 \pm 0.4$  mg/dl, which is much lower than reported in ISIS ( $9 \pm 6$  mg/dl) (ISIS, 2002). However, although BUN in mammals is the main breakdown product of nitrogen metabolism (Duncan et al., 1994), the major breakdown product

in avian species is uric acid. Increases in BUN of avian species have been linked to dehydration (Phalen, 2000). The ibises in this study appeared to be well hydrated and therefore may reflect lower true normal values for the analyzer system used in this study. Elevations of AST and CK may have been related to enzyme leakage from muscle trauma during capture and restraint of some birds, although each bird was netted as quickly (<10 min) and handled as minimally as possible to reduce stress. The remaining biochemical values were within the reference range of most birds.

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