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COMPARISON OF HEMATOLOGIC AND BIOCHEMICAL REFERENCE RANGES BETWEEN CAPTIVE POPULATIONS OF NORTHERN BALD IBISES (*GERONTICUS EREMITA*)

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ABSTRACT: Hematologic and biochemical reference ranges for two captive populations of northern bald ibises (*Geronticus eremita*) were compared. The first consisted of 11 birds at an in-situ breeding colony in Bireçik, southern Turkey. The second consisted of 27 birds housed at the Durrell Wildlife Conservation Trust in Jersey, British Channel Isles (UK). Blood samples were collected in February 1992 by basilic venipuncture under manual restraint. Bireçik birds had higher packed cell volumes and red blood counts but lower white blood cell and lymphocyte counts than Jersey birds. Bireçik birds also had higher total protein, albumin, total globulin, calcium, phosphorus, blood urea nitrogen, and total bilirubin values; higher albumin to globulin ratios; but lower uric acid values and calcium to phosphorus ratios than Jersey birds. Finally, Bireçik birds had higher lactate dehydrogenase but lower gamma glutamyl transferase values than Jersey birds. Male Jersey birds had higher calcium and alkaline phosphatase values, but lower white blood cell and heterophil counts than female Jersey birds. The apparent differences between the two populations are not thought to be biologically significant and may be related to diet and state of hydration.

Key words: *Geronticus eremita,* hematology, northern bald ibis, reference ranges, serum biochemistry.

INTRODUCTION

The northern bald ibis (*Geronticus eremita*), sometimes called the Waldrapp ibis, is a member of the family Threskiornithidae, subfamily Threskiornithinae, which is composed of 26 species of ibises (Del Hoyo et al., 1992). The earliest fossils of Threskiornithidae date back 60 million years to the Eocene; however, this particular species was first described in 1555 by a Swiss physician and naturalist Conrad Gesner (Hirsh, 1977). Its original range included the Near and Middle East, the Alps, southern Europe, and Egypt (Del Hoyo et al., 1992), and protection was assured by European decree in the 16th and 17th centuries (Hirsh, 1977) and through holy legends; the birds were believed to carry the souls of the dead over Mecca during their annual migration (Mallet, 1975). Despite this, the species has been in long-term decline for reasons that include habitat loss, persecution, disease (Del Hoyo et al., 1992), and pesticide use (Collar and Stuart, 1985).

Until a few years ago, the northern bald ibis nested in several colonies in Morocco and Algeria and the south and west Sahara. There was also one colony along the Euphrates River in Bireçik in southeastern Turkey, with birds migrating to Sudan, Eritrea, Ethiopia, Yemen, and Saudi Arabia. A local captive-breeding program, started in 1977, supported this colony. Unfortunately, the Algerian population has subsequently disappeared, numbers are dwindling in Morocco, and the Turkish colony is confined to the captive-breeding facility (Hilton-Taylor, 2000). There are, however, new reports of sightings of this species in Yemen and Saudi Arabia and these may be derived from captive birds released from Bireçik; from other unknown breeding areas in Turkey, Spain, or Iraq; or from new breeding colonies established in the southwest of the Arabian peninsula (Collar et al., 1994). Nevertheless, the northern bald ibis is classified as critically endangered by the International Union for the Conservation of Nature (Hilton-Taylor, 2000) and

is facing a high risk of extinction in the wild in the immediate future.

Fortunately, this species breeds well in captivity. The first recorded breeding colony was established at the Basel Zoo in 1949, the descendants of which are now distributed in breeding centers throughout the world (Archibald et al., 1980). The most recent census (1997) records over 916 individuals in 70 institutions (International Zoo Yearbook, 1998). Jersey Zoo, headquarters of the Durrell Wildlife Conservation Trust (Jersey, UK), has kept northern bald ibis since 1965 (Fidgett and Henry, 1994), and in the period 1975 to 2000, 100 birds have been successfully reared.

In February 1992, a project was undertaken to evaluate the captive colony of northern bald ibises in Bireçik, to monitor their health, to obtain blood samples from these birds for DNA phylogenetic analysis (Tomlinson, 1995), and to establish hematologic and biochemical reference ranges. This project was approved by the Turkish Ministry of Forestry, Department of National Parks, and was arranged with the cooperation of Dogal Hayati Koruma Dernegi (D.H.K.D.), the Society for the Protection of Nature, in Istanbul. For comparison, blood samples were also collected from birds in the Jersey Zoo collection. This paper reports the hematologic and biochemical reference ranges.

MATERIALS AND METHODS

Jersey Zoo is on the island of Jersey (49°13′43″N, 2°4′22″W) in the British Channel Isles, 100 m above sea level. It experiences a typical northern European temperate climate, and in February 1992 had an average air temperature of 6.9 C, total sunshine of 81.8 hr, and 35.2 mm rainfall (Jersey Meteorological Office, pers. comm.). Captive northern bald ibises were housed in two large aviaries, both constructed from wire mesh on a metal framework, with nest boxes, shrubs and suitable branches for perching, a shallow pool for drinking and bathing, and areas of grass, sand, and gravel (Fidgett and Henry, 1994). Birds were fed twice daily a mixed diet that included calf livers and hearts, minced chicks, mealworms, crickets, mice, snails, mixed fruit, dried fruit, turkey breeder pellets, grated carrot, and minced hard-boiled egg (Mallet, 1975). Nesting season was from early March until June or July (Michelmore and Oliver, 1982).

Bireçik is located on the Euphrates River in southeastern Turkey (37°2′9″N, 37°58′57″E), 350 m above sea level. It experiences a typical Mediterranean climate, and in February 1992 had an average air temperature of 2.5 C and rainfall of 85.9 mm (Turkish State Meteorological Office, pers. comm.). Northern bald ibises were housed in two large aviaries set against limestone cliffs. Both were constructed of wire mesh on a wooden framework with a concrete floor. Each incorporated a simple concrete building for shelter and servicing, and the back walls were terraced up to the natural cliff face. Nesting ledges and platforms were provided in the upper part of each aviary. Birds were fed a mixed diet which included lean minced lamb, fishmeal, bonemeal, carrots, lettuce, boiled eggs in their shells, multivitamins, and sea shells. The colony was managed on a semi-liberated basis; the aviaries were opened in late February and resident birds would fly around the region in search of food and nesting material. By August, approximately 50% of the birds had departed, and the aviaries were closed to retain a breeding colony. Birds laid eggs at the end of March; chicks hatched by the end of April. Some birds breed by 2 yr of age and most breed by 3 yr of age. Adults reared their young.

In February 1992, blood was collected from both Jersey and Bireçik birds. Each bird was manually restrained in dorsal recumbency on a padded surface and the area over the basilic vein of the right wing was plucked and cleaned with isopropyl alcohol. One and a half milliliters of blood were collected using a 2 ml syringe with a 25 by 5/8 inch needle. Immediately, 0.75 ml of this blood was transferred to an ethylenediaminetetraacetic acid (EDTA) tube for hematology, and 0.75 ml to a lithium heparin tube for biochemistry. Each tube was gently mixed by repeated inversion for 2 min. Two thin blood films were prepared and air-dried or fixed in 100% methanol for hematology and examination for parasites. At the same time, a cold compress was applied over the vein for 5 min or until hemostasis was achieved. Topical antiseptic dusting powder (Woundcare Powder, Animalcare Ltd., Common Road, Dunnington, York, UK) was applied to the site. The bird was returned quietly to its aviary and briefly observed for any reaction to the handling. Blood samples were stored at approximately 4 C and processed within 4–5 days at Bloxham Laboratories (Axiom Veterinary Laboratories Ltd., Devon, UK). Complete blood cell counts were

TABLE 1. Hematologic and biochemical reference ranges (minimum–maximum [*n*]) for both the Bireçik (B) and Jersey (J) birds and comparisons between the two populations.

Parameter	Bireçik	Jersey	Relationship ($P = 0.05$)
Albumin (g/l)	10.4–13.5 (10)	6.60–11.4 (27)	B > J (E)[a]
Total protein (g/l)	33.4–43.8 (10)	28.8–39.2 (27)	B > J (E)
Total globulins (g/l)	22.6–31.0 (10)	20.8–28.0 (27)	B > J (E)
Albumin:globulin ratio	0.406–0.508 (10)	0.297–0.453 (27)	B > J (MW)[b]
Blood urea nitrogen (mmol/l)	0.300–7.80 (10)	0.400–2.00 (27)	B > J (A)[c]
Creatinine (μmol/l)	36.0–72.0 (10)	26.0–70.0 (27)	B = J (MW)
Total bilirubin (μmol/l)	7.80–17.4 (10)	0.00–1.80 (27)	B > J (K)[d]
Calcium (mmol/l)	2.22–2.79 (10)	2.00–2.38 (27)	B > J (A)
Phosphorus (mmol/l)	1.94–3.33 (10)	0.310–1.64 (27)	B > J (MW)
Calcium:phosphorus ratio	0.721–1.19 (10)	1.42–7.00 (27)	B < J (K)
Gamma glutamyl transferase (IU/l)	0.00–0.00 (10)	0.00–10.6 (27)	B < J (K)
Lactate dehydrogenase (IU/l)	1,010–1,930 (10)	253–893 (27)	B > J (E)
Alkaline phosphatase (IU/l)	57.0–230 (10)	52.0–324 (27)	B = J (MW)
Aspartate aminotransferase (IU/l)	107–183 (10)	87.6–321 (27)	B = J (K)
Alanine aminotransferase (IU/l)	16.8–35.8 (10)	5.50–39.9 (27)	B = J (E)
Uric acid (μmol/l)	256–745 (10)	295–1520 (27)	B < J (MW)
White blood count (10^9/l)	1.00–5.00 (11)	1.80–6.00 (27)	B < J (E)
Red blood count (10^{12}/l)	2.80–3.33 (11)	2.40–3.80 (27)	B > J (K)
Hemogloblin (g/dl)	11.1–15.2 (11)	12.3–15.1 (27)	B = J (K)
Packed cell volume (%)	38.0–50.0 (11)	38.0–48.0 (27)	B > J (E)
Lymphocytes (10^9/l)	0.380–1.02 (11)	0.270–3.08 (27)	B < J (A)
Heterophils (10^9/l)	0.450–4.35 (11)	0.288–4.62 (27)	B = J (E)
Monocytes (10^9/l)	0.020–0.136 (11)	0.00–0.288 (27)	B = J (A)
Basophils (10^9/l)	0.00–0.020 (11)	0.00–0.440 (27)	B = J (K)
Eosinophils (10^9/l)	0.00–0.00 (11)	0.00–0.00 (27)	B = J (K)

[a] Equal-variance *t*-test (E).
[b] Mann-Whitney *U* or Wilcoxon rank-sum test (MW).
[c] Aspin-Welch unequal-variance *t*-test (A).
[d] Kolmogorov-Smirnov test (K).

measured on an automated counter and confirmed manually using a hemocytometer and by examination of the blood smears. Clinical chemistry was performed on an automated analyzer at 30 C.

Only birds considered healthy, based on their weight and physical appearance, were used in this study. Their ages were often unknown, particularly in the Bireçik collection, but in view of the date of sampling, they were all at least 10 mo old. The northern bald ibis is sexually monomorphic and the sex of most Bireçik birds was also unknown.

Hematologic and biochemical reference ranges (minimum and maximum) were calculated for the Bireçik and Jersey birds (Raskin, 2000). Results for each parameter were compared between the two populations and between male and female Jersey birds. Statistical tests included an equal-variance *t*-test, Aspin-Welch unequal-variance *t*-test, Mann-Whitney U or Wilcoxon rank-sum test, or Kolmogorov-Smirnov test (Hintze et al., 1999).

RESULTS

Blood was collected from 11 birds in the Bireçik collection. Unfortunately, a lithium heparin sample was not obtained from one bird in this group. Blood was collected from 27 birds (12 males and 15 females) in the Jersey collection. The hematologic and biochemical reference ranges for the Bireçik and Jersey birds and comparisons between the two populations are shown in Table 1. Comparisons between male and female Jersey birds are shown in Table 2.

DISCUSSION

Hematology and separation of plasma from blood cells was not conducted immediately because of long transit time. This delay could have caused significant alteration in the results of the assays (Fudge,

TABLE 2. Comparisons between 12 male (M) and 15 female (F) Jersey birds.

Parameter	Relationship ($P = 0.05$)
Albumin	M = F (E)[a]
Total proteins	M = F (MW)[b]
Total globulins	M = F (MW)
Albumin:globulin ratio	M = F (MW)
Blood urea nitrogen	M = F (E)
Creatinine	M = F (E)
Total bilirubin	M = F (K)[c]
Calcium	M > F (E)
Phosphorus	M = F (MW)
Calcium:phosphorus ratio	M = F (K)
Gamma glutamyl transferase	M = F (E)
Lactate dehydrogenase	M = F (E)
Alkaline phosphatase	M > F (E)
Aspartate aminotransferase	M = F (MW)
Alanine aminotransferase	M = F (E)
Uric acid	M = F (E)
White blood count	M < F (A)[d]
Red blood count	M = F (MW)
Hemoglobin	M = F (E)
Packed cell volume	M = F (E)
Lymphocytes	M = F (E)
Heterophils	M < F (E)
Monocytes	M = F (E)
Basophils	M = F (K)
Eosinophils	M = F (K)

[a] Equal-variance *t*-test (E).
[b] Mann-Whitney *U* or Wilcoxon rank-sum test (MW).
[c] Kolmogorov-Smirnov test (K).
[d] Aspin-Welch unequal-variance *t*-test (A).

2000a). However, the delay occurred with samples from both groups of birds and so we felt it was legitimate to compare the results.

Hematology and blood biochemistry of birds may vary according to their age, sex, diet, health, geographic location, and egg laying status (Lumeij, 1997). Typically, variation with age occurs in the first few months of life (Montesinos et al., 1997). All birds in this study were at least 10 mo old so age was not considered in the analyses. It was only possible to compare male and female birds from the Jersey collection. Fortunately, there were few differences between the sexes, suggesting minimal influence on subsequent comparisons between the Jersey and Bireçik populations. Male birds had higher calcium and alkaline phosphatase values but lower white blood cell and heterophil counts than female birds. Blood calcium is known to increase in female birds close to ovulation, due to the estrogen-induced transport of yolk proteins to the ovary as calcium complexes (Lumeij, 1997). This was not apparent in the Jersey birds, probably because ovulation was still several weeks away. No difference in alkaline phosphatase values was found between the Jersey and Bireçik birds.

Protein values and packed cell volumes were higher in the Bireçik than in the Jersey birds. One possible explanation for this was relative dehydration within the Bireçik birds, however, both groups of birds had ad libitum access to water and Bireçik had a higher rainfall than Jersey during February 1992. Alternatively, higher altitudes with a reduced oxygen tension can lead to an increased production and release of erythropoietin, which stimulates erythropoiesis and results in an increased PCV, hemoglobin, and red blood count (Jain, 1993; Sturkie and Griminger, 1986). However, since Bireçik was only 250 m higher than Jersey, we felt that this difference in altitude was not significant. The ratios of albumin to total globulins were also higher in the Bireçik than in the Jersey birds.

Uric acid values were higher in the Jersey than in the Bireçik birds. Uric acid is the major end product of nitrogen metabolism in birds, constituting approximately 60 to 80% of the total excreted nitrogen in avian urine. Renal function disorders will eventually lead to increased plasma uric acid concentrations, but only when renal function is below 30% of its original capacity (Lumeij, 1997). In contrast, blood urea nitrogen values were higher in the Bireçik than in the Jersey birds, and this probably reflects differences in handling of these metabolites by the kidney. Blood urea nitrogen is the most useful plasma parameter for early detection of prerenal renal failure (Lumeij, 1997). This is because urea is excreted by glomerular filtration; whereas tubular reabsorption is dependent

on urine flow, which in turn depends on the state of hydration. In general, blood urea nitrogen values are low in birds in comparison to mammals and there is considerable individual variation (Phalen, 2000). Consequently, repeated evaluation over time is more meaningful than a single value.

Bilirubin occurs in scant quantities in avian plasma so bilirubin assays provide no useful clinical information (Fudge, 2000c). It was surprising, therefore, that the total bilirubin values of the Bireçik birds were so high (Henderson, 1997) and perhaps reflects problems with the assay.

Calcium values were slightly higher in the Bireçik than in the Jersey birds. Total calcium concentration is influenced by total protein and albumin concentrations (Lumeij, 1997). Some authors suggest that calcium values should be adjusted according to protein concentrations (Lumeij, 1997) while others disagree (Fudge, 2000b). Since albumin values were also higher in the Bireçik than in the Jersey birds, this may help to explain the difference in the calcium values. Bireçik birds also had higher phosphorus values but lower calcium to phosphorus ratios than Jersey birds. These differences are most likely related to diet.

The packed cell volumes and red blood counts were higher in the Bireçik than in the Jersey birds. The white blood cell and lymphocyte counts were lower in the Bireçik than in the Jersey birds. Lymphopenia is associated with endogenous corticosteroid release, associated with restraint, starvation, shipping, fear, and excitement (Fudge and Joseph, 2000). It is possible that the Bireçik birds were less acclimatized to human interactions than Jersey birds, and therefore developed a stress lymphopenia during handling, resulting in lower white blood counts.

These data provide reference ranges for northern bald ibises. They will allow better evaluation of the health status of this species in the future.

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