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ABSTRACT: Hematological and plasma chemistry parameters were measured in 129 juvenile white storks (*Ciconia ciconia*), either wild or captive bred, April to June 1994. Wild storks were members of a colony in the Lozoya River Valley, Madrid, Spain. Red blood cells count, packed cell volume and hemoglobin increased significantly with age. White blood cells count, lymphocytes count and platelets decreased with age. Total solids, total proteins, fibrinogen, albumin, alpha, beta, gamma-globulins and urea increased with age. Differences between captive and wild birds were not notable.

Key words: White stork, *Ciconia ciconia*, hematology, plasma chemistry.

INTRODUCTION

White storks (*Ciconia ciconia*) commonly nest near humans, in urban settings. Due to the alarming decrease of the stork population in past decades, these birds became the focus of various conservation programs in Spain and the rest of Europe, and, as a result, their numbers have risen in recent years. Many of these programs have been carried out by avian recuperation centers sponsored by local or state governments. At present, the white stork is no longer considered a threatened species in Spain.

One of the principal tasks undertaken by these recuperation centers has been to establish rehabilitation programs for wounded and sick birds. Most storks which receive veterinary attention in these centers are injured fledglings and ailing chicks found in their nests by members of ornithological societies who band the birds. The storks' adaptation to the new urban environments and the loss of former wetlands have been related to the introduction of new diseases and an increase in previously known problems such as collisions with electric power lines. An ever-growing number of young storks are found with gastrointestinal impactions caused by strings and cables which the birds may mistake for worms or snakes. Modifications in feeding habitats also may have led to the concentration of birds in rubbish dumps, increasing the risk of collision with power lines and impactions.

Hematology and blood biochemistry form a part of the diagnostic process of all birds, including young storks, brought to the recuperation centers (Dein, 1986; Hawkey and Samour, 1988). Our study was designed to determine the hematological and serum biochemical reference intervals in both young wild storks and those born in captivity.

MATERIALS AND METHODS

We analyzed 129 blood samples taken from juvenile white storks (*Ciconia ciconia*). The seven storks hatched in the Cenicientos (4°30'N, 40°15'W), Madrid Recuperation Centre, in 1994 were kept in Animal Intensive Care Unit (AICU)-type avian incubators (Animal Care Products, Norko, California, USA) for their first month of life and were hand-fed, employing a puppet representing an adult stork, until they were old enough to eat alone. The eggs came from nests whose occupants were known to have died, usually from gunshot wounds or during repairs of the church roofs where the nests were built. The eggs thus obtained were kept in standard avian incubators until the chicks hatched. After reaching 1 mo of age, the chicks were housed with other storks in artificial nests. The birds were not visually exposed to humans during their growth period and reached flying age without imprinting problems. All the birds admitted into the Centre were routinely dewormed with fenbendazol (Panacur®, Hoechst-Roussell, Paris, France). Blood samples were obtained from

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the birds hatched in captivity within the first 72 hr after hatching and subsequently on days 15, 30, 45 and 90.

The blood samples from young white storks in the wild were obtained from members of a colony in the Lozoya River Valley (Community of Madrid) (3°45′N, 40°55′W). Members of the Spanish Ornithological Society were conducting a study of these birds during their nesting season. The storks belonged to a colony of 36 nests built in easily reached ash trees (*Fraxinus excelsior*). This colony was under surveillance by ornithologists who also recorded the age of the young birds. The study began with 31 chicks, but that number decreased, due primarily to gastrointestinal impactions and abandonment on the part of some parents. Blood samples were taken from individuals in the same nests and were classified into three groups according to the age of the chicks: under 12 days of age ($n = 31$), between 17 and 32 days-old ($n = 25$) and between 44 and 52 days of age ($n = 23$).

Our study lasted from the end of April to the beginning of June 1994. No differentiation was made between the sexes of the storks whose blood was studied as these birds do not exhibit sexual dimorphism.

The blood was obtained from the right jugular vein by means of 3 ml syringes and 25 G needles. Both the birds hatched in captivity as well as those found in nests were manually restrained while the samples were collected. The blood was carefully transferred to test tubes containing an anticoagulant; 0.75 ml of the sample were combined with dipotassium EDTA and the rest (1.25 ml) was placed in heparinized test tubes. Smears were prepared at once and methanol (3 min) was used as a fixative at the time of the extraction. The test tubes with the blood were stored between 0 and 4 C until they reached the laboratory. The blood taken from the storks in captivity was processed immediately upon extraction. The blood samples from the wild storks reached the laboratory 6 to 12 hr after collection.

For the red and white cell counts, the whole blood was diluted 200 and 50 times, respectively, using Natt and Herrick's solution, in blood-cell dilution pipettes (Campbell, 1988). Using an improved Neubauer hemocytometer, the red blood cells seen in the 10 groups of 16 small squares, and all the big cells (white blood cells) seen in the Neubauer slide were counted. The hemoglobin content was determined using the Drabkin technique (Drabkin, 1945) modified by the addition of distilled water to the hemolytic agent.

Blood smears were stained with May-Grünwald Giemsa stains (Campbell, 1988). At least 400 cells were counted in each smear; the absolute number of thrombocytes was determined by comparing their number with the total white cell count. No attempt was made to identify immature erythrocytes or to differentiate between large and small lymphocytes. The appropriate formulas were used to determine mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values (Campbell, 1988).

The biochemical plasma analyses were undertaken using a dry chemical system (Reflotron®, Boehringer-Manheim, Barcelona, Spain) (Schwendenwein, 1988). The choice of the parameters studied was made taking into account their clinical utility, the availability of the means needed for the analyses and economic considerations.

Refractometry at room temperature (21 C) was used to estimate the total plasma solids. Electrophoresis of the plasma proteins was performed on cellulose acetate, using the heparinized plasma (Dein, 1986; Campbell, 1988). The total albumin reading has been separated into the albumin and prealbumin fractions, in order to check whether the latter index decreased in the chicks with age. Globulin values were determined by adding up the alpha, beta, and gamma globulin fractions. Fibrinogen content was computed using the heat microprecipitation technique at 56 C for 3 min on the same sample employed to ascertain the hematocrit (Campbell, 1988). The total proteins/fibrinogen ratio was worked out using the proper formula (Campbell, 1988).

Statistical analysis was performed using the computer program SIGMA (Horus Hardware, Madrid, Spain). An analysis of variance (ANOVA) was used to determine significant differences between groups. Values of $P \leq 0.01$ were considered statistically significant.

RESULTS

Most hematological values in wild stork chicks varied significantly with age (Table 1). Of these, red blood cells count, packed cell volume, hemoglobin and heterophil: lymphocyte ratio increased significantly with age. White blood cells count, lymphocytes count and platelets decreased with age. All of the biochemical parameters studied in wild storks chicks varied significantly with age except triglycerides, cholesterol, uric acid, potassium, proteins : fibrinogen ratio and albumin : globulins ratio (Table 2). Total solids, total proteins, fibrinogen, albumin, alpha, beta, gamma-

TABLE 1. Hematological values in wild stork chicks from Lozoya River Valley, 1994.

| | Age of chicks | | | | | |
| | <12 days (n = 31) | | 17–32 days (n = 25) | | 44–56 days (n = 23) | |
Hematological parameters	Mean	SD	Mean	SD	Mean	SD
Packed cell volume (%)[a]	25.1	0.4	34.6	0.5	40.1	0.4
Hemoglobin (g/l)[a]	102.2	8.1	109.5	14.1	126.5	7.9
Red blood cells (×10^{12}/l)[a]	1.16	0.17	1.49	0.25	2.19	0.30
Mean corpuscular volume (fl)[a]	226.3	30.4	233.4	32.4	190.2	23.1
Mean corpuscular hemoglobin (pg)[a]	90.1	6.7	72.7	8.9	57.2	6.1
Mean corpuscular hemoglobin concentration (g/l)[a]	404.0	10.5	311.3	15.4	310.7	15.9
Thrombocytes (×10^9/l)[a]	65.90	6.75	46.52	5.77	29.2	5.09
White blood cells (×10^9/l)[a]	38.78	2.87	31.08	1.46	24.54	2.82
Heterophils (×10^9/l)	20.09	0.85	21.12	1.24	19.48	0.80
Lymphocytes (×10^9/l)[a]	14.74	1.53	7.16	0.77	3.07	0.47
Monocytes (×10^9/l)	0.30	0.30	0.40	0.23	0.22	0.22
Eosinophils (×10^9/l)	1.95	0.57	1.45	0.23	1.22	0.47
Basophils (×10^9/l)	0.16	0.16	0.10	0.10	0.04	0.04
Heterophil : lymphocyte ratio[a]	1.36	0.20	2.97	0.42	6.34	1.26

[a] Values significantly different among all the groups (P < 0.01) (ANOVA).

globulins and urea increased and aspartate amino-transferase decreased with age.

Most wild birds, as well as those hatched in captivity, had age-related variations in hematological parameters (Table 3). The packed cell volume, hemoglobin, red blood cell count and heterophil : lymphocyte ratio also rose with age in the wild birds group. Total leucocytes and thrombocytes and the percentage of lymphocytes and eosinophils decreased with age.

With regard to the biochemical parameters, the total solids, total proteins, fibrinogen, electrophoretic fractions, urea, cholesterol and aspartate amino-transferase increased with age (Table 4). In contrast, uric acid decreased and the other values displayed no variation.

Among birds treated in the Centre that were born in the same year but already were flying at the time the samples were taken, no important differences were found between hematological and biochemical values for birds > 90 days old and those ≤ 90 days (Table 5). They had been taken to the Centre for various reasons and the values refer to the last blood sample before they were released. In the storks hatched in captivity as well as in the

wild birds, the age of the first flight ranged from 55 to 70 days after hatching.

DISCUSSION

The hematology and blood biochemistry of birds may vary according to the geographical area, diet, state of health, handling and care in general (Lumeij and Bruijne, 1985; Fowler, 1986). As also occurs in mammals, the values of young birds may vary significantly from those of adults (Hawkey et al. 1984; Drew et al., 1993). We found that many of the stork's hematological parameters differed significantly in accordance with their age and that many of these variations are similar to those reported in other species of birds (Clubb et al., 1991).

Storks begin to fly at about 70 days of age. In this study the birds hatched in captivity were kept under control until they were 90 days old, at which time they were already flying. When one compares the hematological values corresponding to 90-day-old birds, published values for adult birds, and those which we obtained from young storks treated in the Centre (all of which were born in the year of the study), scarcely any variation was seen,

TABLE 2.   Biochemical values in wild stork chicks from Lozoya River Valley, 1994.

| Biochemical parameters | Age of chicks | | | | | |
| | <12 days (n = 31) | | 17–32 days (n = 25) | | 44–56 days (n = 23) | |
	Mean	SD	Mean	SD	Mean	SD
Total solids (g/l)[a]	21.3	1.1	29.0	1.4	36.5	0.6
Total proteins (g/l)[a]	20.9	0.9	24.6	2.1	30.4	0.4
Fibrinogen (g/l)[a]	4.1	0.9	5.1	2.1	6.0	2.4
Total proteins : fibrinogen (ratio)	5.1	0.3	4.8	0.6	5.1	0.5
Albumin (g/l)[a]	9.4	0.4	11.3	0.6	13.9	0.7
Prealbumin (g/l)[a]	0.4	0.1	1.2	0.2	3.8	1.2
Alpha-globulins (g/l)[a]	4.4	0.3	4.5	0.2	5.0	0.5
Beta-globulins (g/l)[a]	2.8	0.2	5.1	0.3	5.2	0.5
Gamma-globulins (g/l)[a]	4.8	0.5	5.0	0.7	7.0	0.2
Globulins (g/l)[a]	11.9	0.2	14.5	0.4	17.3	0.3
Albumin : globulins (ratio)	0.79	0.1	0.77	0.2	0.80	0.1
Triglycerides (mmol/l)	2.1	0.2	2.2	0.2	1.9	0.3
Cholesterol (mmol/l)	4.5	0.7	4.8	0.3	4.9	0.9
Uric acid (μmol/l)	863.4	171.6	767.9	129.9	801.7	184.8
Aspartate amino-transferase (AST) (IU/l)[a]	350	22.3	245	19.4	182.9	21.9
Creatine kinase (CK) (IU/l)[a]	229	65.3	304	61.1	259.6	80.4
Glucose (mmol/l)	13.2	1.5	14.2	0.8	13.7	0.6
Urea (mmol/l)[a]	3.9	0.5	4.9	0.3	5.2	0.6
Potassium (mmol/l)	4.4	0.4	4.5	0.4	4.4	0.2

[a] Values significantly different among all the groups (P < 0.01) (ANOVA).

even in premigratory birds; however, the number of birds tested by us may not be statistically significant. These birds had a typical adult hemogram after 80 to 90 days of age.

Results of our red blood cell counts and other related parameters were similar to those obtained by other authors who studied stork chicks (Puerta et al. 1989), but our study was unique in that other investigators studied a colony at one particular moment in time, without any knowledge over the age of the birds tested. As is seen in the age-determined subgroups of captive and wild storks in this study (Tables 1, 3 and 5), age-related variations of the red blood cells count, hemoglobin and packed cell volume occurred. These variations were probably due to the preparation of the birds for flight, at which time the need for oxygen was greatly increased (Hawkey et al., 1984).

The total and differential white blood cell counts are routinely used as an index of illness in mammals and birds (Dein,

1986; Campbell, 1994). Comparing the total and differential white cell counts obtained in this study with data previously published, we believe that important differences in the results exist. Puerta et al. (1989) cited extremely high total white cell counts of more than 60,000/μl and noted lymphocytes as the predominant cells. We did not find numbers as high nor did our findings coincide as to the predominant cell type. The findings of our study were more in line with the adult bird hemogram described by Hawkey and Samour (1988), although we found that the youngest birds presented the highest counts and that these decreased with age. We saw heterophilia and high eosinophil values in the birds, although not as high as those reported by Hawkey and Samour (1988). When it was possible to check for parasites in the feces (birds born in the Centre or treated for diverse reasons), no relationship was found between the number of eosinophils and the presence of parasites

TABLE 3. Hematological values in stork chicks born in captivity from Cenicientos in 1994 ($n = 7$ for birds < 1 wk old; $n = 6$ for the others).

Hematological parameters	Age of chicks									
	<1 week		15 days		30 days		45 days		90 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Packed cell volume (%)[a]	25.0	3.9	32.0	2.8	36.1	2.5	41.5	2.8	43.0	2.1
Hemoglobin (g/l)[a]	91.9	4.2	114.2	7.7	115.7	18.6	128.8	22.3	131.3	21.5
Red blood cells (×10^{12}/l)[a]	1.14	0.21	1.22	0.26	1.59	0.45	1.87	0.24	1.84	0.20
Mean corpuscular volume (fl)[a]	220.0	24.5	262.6	27.4	225.7	20.1	222.0	16.5	237.0	21.2
Mean corpuscular hemoglobin (pg)[a]	82.0	2.9	91.3	3.7	73.8	8.1	68.3	2.6	71.4	8.2
Mean corpuscular hemoglobin concentration (g/l)[a]	364.4	40.3	351.7	10.3	315.5	16.2	311.5	10.6	311.2	15.2
Thrombocytes (×10^9/l)[a]	73.00	7.96	61.17	8.08	44.52	7.20	27.00	4.47	22.00	1.41
White blood cells (×10^9/l)[a]	40.10	1.33	36.73	3.08	30.72	1.04	25.38	1.61	23.88	2.78
Heterophils (×10^9/l)	17.93	2.04	17.94	1.88	18.43	1.70	19.80	0.74	18.43	1.24
Lymphocytes (×10^9/l)[a]	18.39	1.93	12.86	1.33	7.68	0.61	3.38	0.55	4.22	1.32
Monocytes (×10^9/l)	0.46	0.36	0.53	0.49	0.51	0.46	0.55	0.37	0.32	0.25
Eosinophils (×10^9/l)[a]	3.21	1.37	2.82	0.50	2.66	0.66	1.57	0.63	1.71	0.55
Basophils (×10^9/l)	0.17	0.14	0.06	0.06	0.05	0.05	0.04	0.04	0.04	0.04
Heterophils : lymphocytes (ratio)[a]	0.97	0.22	1.39	0.30	3.40	0.41	5.86	1.20	4.37	1.84

[a] Values significantly different among all the groups ($P < 0.01$) (ANOVA).

TABLE 4. Biochemical plasma values in stork chicks born in captivity from Cenicientos in 1994 ($n = 7$ for birds <1 wk old, $n = 6$ for the others).

Biochemical parameters	Age of chicks									
	<1 week		15 days		30 days		45 days		90 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total solids (g/l)[a]	18.0	2.1	28.5	1.1	30.2	1.5	35.3	0.6	36.0	0.6
Total proteins (g/l)[a]	14.1	1.3	22.0	1.4	24.0	1.4	29.5	0.5	30.2	0.6
Fibrinogen (g/l)[a]	3.5	0.7	5.0	0.6	5.5	2.9	6.0	2.0	6.0	0.6
Total proteins : fibrinogen (ratio)	4.0	0.3	4.4	0.4	4.4	0.9	4.9	0.6	5.0	0.5
Albumin (g/l)[a]	5.8	0.4	10.0	1.5	11.3	1.1	13.3	1.1	13.2	1.6
Prealbumin (g/l)[a]	0.8	0.1	3.2	0.7	2.2	0.2	4.3	0.4	2.9	0.6
Alpha-globulins (g/l)[a]	2.4	0.4	4.2	1.0	5.0	0.7	5.3	1.6	5.5	1.2
Beta-globulins (g/l)[a]	1.9	0.4	3.2	0.1	2.7	0.9	4.5	0.7	4.6	0.7
Gamma-globulins (g/l)[a]	3.5	0.5	5.7	1.2	6.3	1.2	7.3	0.5	7.2	1.2
Globulins (g/l)[a]	7.7	0.7	12.8	1.6	13.9	1.5	16.8	1.6	17.3	1.4
Albumin : globulins (ratio)	0.75	0.1	0.78	0.2	0.81	0.1	0.79	0.1	0.76	0.1
Triglycerides (mmol/l)	1.1	0.3	0.9	0.3	1.1	0.2	1.3	0.2	1.2	0.2
Cholesterol (mmol/l)[a]	2.7	0.6	3.7	0.4	4.8	0.4	5.0	0.5	4.8	0.5
Uric acid (μmol/l)[a]	1,107.3	114.1	737.1	107.3	612.2	94.9	669.7	63.1	647.9	60.3
Aspartate amino-transferase (AST) (IU/l)[a]	190.7	18.4	293.8	11.8	195.7	10.9	205.2	13.6	393.0	11.5
Creatine kinase (CK) (IU/l)	214.5	61.8	224.0	71.1	198.5	60.3	213.7	39.3	203.5	39.0
Glucose (mmol/l)	12.8	0.9	12.8	1.0	13.0	0.5	12.0	1.3	11.8	0.5
Urea (mmol/l)[a]	2.5	0.4	3.7	0.4	4.3	0.5	7.2	0.6	6.4	0.7
Potassium (mmol/l)	3.8	0.1	3.8	0.2	4.1	0.1	4.2	0.1	4.3	0.2

[a] Values significantly different among all the groups ($P < 0.01$) (ANOVA).

TABLE 5. Hematological and biochemical values in birds more than 90 days old that were born in the year of the study but treated in the Centre. The values correspond with the day before the birds were set free in the wild, when they were considered to be healthy ($n = 19$ for all parameters).

Parameters	Mean	SD	Parameters	Mean	SD
Packed cell volume (%)	41.2	2.0	Total solids (g/l)	36.0	0.9
Hemoglobin (g/l)	142.0	5.9	Total proteins (g/l)	30.9	0.4
Red blood cells ($\times 10^{12}$/l)	2.2	0.3	Fibrinogen (g/l)	5.6	1.1
Mean corpuscular volume (fl)	191.9	8.4	Albumin (g/l)	13.5	0.7
Mean corpuscular hemoglobin (pg)	62.5	2.8	Prealbumin (g/l)	4.4	0.6
Mean corpuscular hemoglobin concentration (g/l)	328.3	11.3	Alpha-globulins (g/l)	4.9	0.6
			Beta-globulins (g/l)	3.6	1.1
Thrombocytes ($\times 10^9$/l)	21.74	3.21	Gamma-globulins (g/l)	6.3	1.3
White blood cells ($\times 10^9$/l)	25.36	3.03	Globulins (g/l)	15.9	0.6
Heterophils ($\times 10^9$/l)	18.12	1.56	Albumin : globulins (ratio)	0.85	0.2
Lymphocytes ($\times 10^9$/l)	4.92	1.21	Triglycerides (mmol/l)	1.2	0.1
Monocytes ($\times 10^9$/l)	0.64	0.40	Cholesterol (mmol/l)	5.6	0.3
Eosinophils ($\times 10^9$/l)	1.86	0.41	Uric acid (μmol/l)	693.3	99.3
Basophils ($\times 10^9$/l)	0.04	0.04	Aspartate amino-transferase (IU/l)	330.3	22.4
Heterophils : lymphocytes (ratio)	3.7	0.9			
Glucose (mmol/l)	13.64	1.37	Urea (mmol/l)	6.7	0.9
Potassium (mmol/l)	4.1	0.2	Alanine amino-transferase (IU/l)	18.4	4.8

eggs or worms expelled as a result of antihelmintic treatments.

The primary reason for our differences with Puerta et al's (1989) results may be due to differences in methodology employed in each study. As with Hawkey et al. (1984) and Hawkey and Samour (1988), we used the May-Grünwald-Giemsa stain to study the smears; we consider it the best for differentiating the staining characteristics of the cells. We believe the difference in the counts was due to the anticoagulant. We used dipotassium EDTA because, even though it can cause hemolysis in some cranes (*Grus antigone* and *Grus monacha*), Corvidae (*Urocissa erythrorhynca*, *Pica pica*, *Cissa chinensis* and *Corvus corax*), kookaburras (*Dacelo novaeguineae*) and great owls (*Bubo bubo*) (Hawkey and Samour, 1988); no such effect is reported in storks and we did not observe this phenomenon. Various authors recommend this substance as the anticoagulant of choice for hematological studies. Heparin is not recommended as it causes thrombocytic and leucocytic clusters which make the total count unreliable (Hawkey et al. 1984, Hawkey and Samour, 1988).

The biochemical data observed in our birds were similar to the values observed by other authors (Hawkey and Samour, 1988; Puerta et al., 1989). We observed slight differences between the uric acid and triglycerides indices of storks hatched in captivity and those in the wild, which we attribute to the lack of control over the eating times of the wild storks; in the case of the birds born in captivity the samples were taken before the first meal of the day, on an empty stomach. We also saw a slight variation in the potassium values (Table 4), possibly due to the time which elapsed between blood collection and processing in the case of the wild birds (Lumeij and Bruijne, 1985; Lumeij and Overdeen, 1990).

The criteria used to choose parameters evaluated depended on technical and economic considerations. A complete hemo-gram, plasma biochemistry (aspartate amino-transferase, creatine kinase, uric acid and proteins values) and fecal analysis were carried out in all the birds treated in the Centre. Depending upon the results and in order to aid diagnosis, other parameters sometimes were evaluated. We attempted to measure the greatest possible number of clinical parameters useful in avian medicine and feasible with the dry chemical system (Reflotron®). We also used electrophoresis to separate the plasmatic protein fractions as this is considered useful for following the evolution of chronic inflammatory diseases (Hochleithmer, 1994). We employed heparinized plasma, however, following the advice of other investigators who recommend its use for this kind of clinical analysis, especially in small birds from which only a limited quantity of blood can be collected (Hochleithmer, 1994).

In this study we found that the hematological and biochemical values of young storks had notable variations in relation to the parameters of adults. This fact should be considered when these birds receive veterinary care.

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