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HEMATOLOGICAL AND SERUM CHEMISTRY NORMS FOR SANDHILL AND WHOOPING CRANES

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HEMATOLOGICAL AND SERUM CHEMISTRY NORMS FOR SANDHILL AND WHOOPING CRANES

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Abstract: The normal values used as a diagnostic tool and for comparison of cranes were established in the early 1970's. In that early study, no effort was made to look at factors such as age, sex, or subspecies. In addition, during the early study disease problems (primarily disseminated visceral coccidiosis) and nutritional problems were undiagnosed and uncontrolled. For 2 years during the annual health examinations of cranes at the USGS Patuxent Wildlife Research Center (Patuxent), we collected blood from healthy cranes for analysis. We found significant differences between the values reported from the 1970's and the values seen in this study for 8 blood parameters for Florida sandhill cranes (*Grus canadensis pratensis*), 6 blood parameters for greater sandhill cranes (*G. c. tabida*), and 6 blood parameters for whooping cranes (*Grus americana*). In addition, there were significant differences for some hematology and serum chemistry values based on the age of the cranes.

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Key words: blood counts, clinical pathology, cranes, Grus americana, Grus canadensis, hematology, serum chemistry.

The current program to breed endangered cranes at Patuxent was established by the Bureau of Sport Fisheries and Wildlife expressly for the scientific study and propagation of species "whose survival could not be assured by existing methods" (Erickson 1968). The program currently houses 40 endangered whooping cranes for breeding purposes plus approximately 25–30 juveniles awaiting release to the wild. In addition, 80–90 Florida sandhill cranes and a similar number of greater sandhill cranes are housed at the research center for use in various scientific studies to benefit the endangered whooping crane population.

The normal clinical pathology values (hematology and serum chemistries) used for comparison today are based on values obtained in 1973–75 at Patuxent by Gee et al. (1981). The results from that study were based on only 15 whooping cranes and 15–20 sandhill cranes (but no effort was made to identify or separate the subspecies of sandhill cranes). Although the cranes used in the 1973–75 study appeared to be in relatively good health, we now know that there were serious disease problems (primarily disseminated visceral coccidiosis, Carpenter et al. 1979, 1980, 1984) and nutritional problems (Serafin 1982) in the captive cranes at that time. Since 1975, several diseases present in the captive cranes have been identified and controlled (Carpenter et al. 1979, 1980, 1984, Olsen and Carpenter 1997). In addition, a major nutritional study conducted in the late 1970's (Serafin 1982) identified dietary problems that have since been corrected. Also, since 1975, many advancements have been made in developing more precise methods for measuring clinical serum chemistry values. All of these factors led to this 2-year study to identify new standard values for blood parameters in captive cranes.

Several additional hematological and serum chemistry tabulations have been done since 1975. A study completed by Carpenter (1986) reported several important species differences between whooping cranes and sandhill cranes. A second compilation of blood parameters (Olsen et al. 1996) produced a table of normal hematologic and serum chemistry values for healthy cranes of 6 different species and pediatric values for sandhill cranes, all held in captivity at Patuxent or the International Crane Foundation, Baraboo, Wisconsin, USA. Other than looking at pediatric values, no study has focused on age-related differences in cranes, yet we now have a population ranging in age from 0-37. Age-related differences could be important in diagnosing and monitoring disease conditions in cranes of varying ages.

METHODS

Standard methods (Dein 1984, Olsen et al. 1996) were used to collect blood samples from clinically healthy cranes during the annual health examinations conducted at Patuxent during September through November, 1996 and 1997, and from healthy young whooping cranes being tested for release (October 1996 through January 1997 and October 1997 through January 1998). Blood samples were collected from

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Proc. North Am. Crane Workshop 8:2001

the right jugular vein using sterile techniques and were stored until processed in Becton Dickinson vacutainers (models 6510-6A901, 6385-5B155, or 6388-5C011, Becton Dickinson VACUTAINER Systems, Franklin Lakes, New Jersey, 07417-1885, USA). Blood smears for "differentials" were made using the 2-cover-slip technique (Dein 1984) and stained with Diff Quick (Jorgensen Laboratories, Loveland, Colorado 80538, USA). White blood cell counts were calculated from a heterophyll/eosinophil count performed using a Becton Dickinson Eosinopil Unopette (#5877, Becton Dickinson) and corrected with the results from the differential count. Serum chemistry values were run on a Dupont Chemistry Analyzer (Dupont, Medical Products Department, Wilmington, Delaware, 09898, USA) or were run by an outside commercial laboratory (Antech Diagnostics, Farmingdale, New York, 11735, USA). Some samples were divided in half and serum chemistry was performed both at Patuxent and by the commercial laboratory to check for accuracy.

Significant differences among the 3 crane groups were detected using Tukey's studentized range test under the general linear model procedure of SAS (SAS Institute, Inc. 1988). To compare the means from the 1973–75 study (Gee et al. 1981) with the results from this study, a Student's t-test was used. Age related differences were detected using a

Table 1. Means and standard deviations of blood parameters for captive Florida sandhill cranes, greater sandhill cranes, and whooping cranes at USGS Patuxent Wildlife Research Center with a comparison of values among the 3 crane groups (Tukey test, see footnote).

	Florida	Sandhill C	ranes	Greater	Sandhill Cr	anes	Wh	ooping Cran	es
Variable	Mean	SD	n	Mean	SD	n	Mean	SD	n
Heterophils (%)	66.4ª	11.0	50	64.1ª	5.2	33	63.7ª	11.7	40
Lymphocytes (%)	31.1ª	11.1	50	34.2ª	5.1	33	32.3ª	9.3	40
Monocytes (%)	1.0ª	1.2	50	0.5ª	0.6	33	1.2ª	1.8	40
Eosinophils (%)	1.3ª	1.0	50	1.2ª	1.1	33	2.6ª	3.5	40
H/E count (No./mm ³)	8185ª	3185	50	6262ª	1893	30	9467⁵	2570	40
White blood cells (No./mm ³)	11968ª	3705	50	9610ª	2863	30	14472 ^ъ	4114	40
Hematocrit (%)	36.9ª	6.4	50	3 9.1ª	3.6	30	39.6ª	3.5	40
Total solids (g/dl)	3.9ª	0.5	50	4.1ª	0.5	30	4.2ª	0.4	40
Glucose (mg/dl)	252ª	24	50	248ª	34	33	241ª	29	40
Total protein (g/dl)	3.0ª	0.4	50	3.0ª	0.4	33	3.3 ^b	0.4	40
Albumin (g/dl)	1.4ª	0.2	50	1.4ª	0.1	33	1.5 ^b	0.2	40
Globulin (g/dl)	1.7ª	0.3	50	1.6ª	0.3	33	1.8ª	0.3	40
Albumin/globulin	0.85ª	0.20	50	0.94ª	0.16	33	0.89ª	0.18	40
Alkaline phosphatase (IU/l)	479ª	334	50	371ª	190	33	169 ^b	78	40
Lactic dehydrogenase (IU/l)	537ª	341	43	234 ^b	83	28	374ª	123	33
Creatine kinase (IU/l)	1784ª	2171	49	284 [⊾]	129	30	375 [⊾]	250	39
Aspartase aminotransferase (IU/I)	196ª	53	50	195 °	39	33	294 [⊾]	68	39
Calcium (mg/dl)	9.96ª	0.91	50	9.19ª	0.73	33	9.17ª	0.53	40
Phosphorus (mg/dl)	4.12ª	1.52	50	3.65ª	1.68	33	3.52ª	1.33	39
Uric acid (mg/dl)	9.49ª	3.13	50	6.13 ^b	2.58	33	5.68⁵	2.24	40

a,b,c Means with the same letter superscript in any row are not significantly different (alpha = 0.05).

Proc. North Am. Crane Workshop 8:2001

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	Curi	rent	Histo				
Variable	Vai	ues	Valu	Values*			
	Mean	n	Mean	n			
Hematocrit (%)	39.0	30	44.0	20	5.93°		
Glucose (mg/dl)	255	27	229	18	-2.67°		
Total protein (g/dl)	3.06	27	3.90	17	7.09°		
Albumin (g/dl)	1.39	27	1.40	18	0.19		
Alkaline phosphatase (IU/l)	248.2	26	184.0	18	-1.67		
Lactic dehydrogenase (IU/1)	267.3	26	257.0	18	0.38		
Calcium (mg/dl)	9.23	27	9.80	18	3.64°		
Phosphorus (mg/dl)	3.62	27	3.60	18	0.01		
Globulin (g/dl)	1.67	27	2.30	18	-8.09°		
Albumin/globulin	0.85	27	0.56	18	8.49°		
Uric acid (mg/dl)	7.83	27	9.70	16	4.29°		

Table 2. A comparison of blood parameters for Florida sandhillcranes between current and historic values at USGS PatuxentWildlife Research Center using t-tests to compare means.

Table 3. A comparison of blood parameters for greater sandhill
cranes between current and historic values at USGS Patuxent
Wildlife Research Center using t-tests to compare means.

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Variable	Valu	ies	Valu	tb	
	Mean	n	Mean	n	
Hematocrit (%)	38.0	30	41.0	18	2.46°
Glucose (mg/dl)	246	28	264	20	1.49
Fotal protein (g/dl)	2.95	28	3.90	20	4.29°
Albumin (g/dl)	1.43	28	1.4	20	1.25
Alkaline phosphatase [IU/I)	410.8	27	146.0	20	-1.14
Lactic dehydrogenase TU/I)	239	28	296	20	2.34°
Calcium (mg/dl)	9.3	28	9.6	20	1.95
Phosphorus (mg/dl)	3.77	28	3.70	20	0.05
Globulin (g/dl)	1.51	28	2.30	20	-5.06°
Albumin/globulin	0.96	28	0.69	20	8.21°
Uric acid (mg/dl)	5.98	28	9.70	19	1.74°

* Historic Values are from Gee et al. (1981).

b t = student's t value.

^c Significance was assumed if P < 0.05.

2-way analysis of variance test (ANOVA). Cranes were grouped by age to increase the sample sizes in each category. The 5 age groups were young-of-the-year, 1-year-old, 2–5years-old, 6–15-years-old, and >15-years-old. The oldest cranes in this study were 35 years old. The age groups were chosen to mirror the life histories of the cranes. Young-ofthe-year show juvenile features. One-year-old cranes or yearlings still have some juvenile characteristics but are mature in size, although not sexually mature. Cranes are mature sexually when 2–6 years of age. Cranes 7–15-yearsold are the mature breeding cranes generally, and cranes older than 15 years, while still active breeders, may show evidence of aging.

RESULTS

When comparing the mean results of our study, there were significant differences between whooping cranes and sandhill cranes (either the Florida or greater subspecies) for white blood cell count, total protein, albumin, lactic dehydrogenase, and aspertase aminotransferase (Table 1).

There were significant differences between whooping cranes and greater sandhill cranes for globulin and alkaline * Historic values from Gee et al. (1981).

^b t = student's t value.

^c Significance was assumed if P < 0.05.

phosphatase. Whooping cranes and Florida sandhill cranes had significantly different mean values for uric acid. Finally, Florida and greater sandhill cranes had significantly different values for alkaline phosphatase, creatinine phosphokinase, and uric acid.

Florida sandhill cranes were found to have significantly different values in this study as compared to the Gee et al. (1981) study for hematocrit, glucose, total protein, calcium, globulin, albumin/globulin ratio, and uric acid (Table 2). Greater sandhill cranes showed a similar pattern of differences between this study and Gee et al.(1981). Differences were seen with hematocrit, total protein, lactic dehydrogenase, globulin, albumin/globulin ratio, and uric acid (Table 3).

Whooping crane blood values also significantly varied between this study and the Gee et al. (1981) study. Significant differences were seen in values for hematocrit, total protein, alkaline phosphatase, globulin, albumin/globulin ratio, and uric acid (Table 4).

Examining differences between cranes of various ages, we found significant differences (P < 0.05) for hematocrit, alkaline phosphatase, aspartate aminotransferase, calcium, uric acid, lactic dehydrogenase, and creatinine kinase for

Florida sandhill cranes (Table 5). Similar age-related differences were seen for greater sandhill cranes for hematocrit, glucose, aspartate aminotransferase, calcium, phosphorus, albumin/globulin ratio, globulin, and creatine kinase (Table 6). Whooping cranes showed the least number of differences in hematology and serum chemistry based on age, with only hematocrit and alkaline phosphatase having significant differences (P < 0.05) based on age (Table 7).

DISCUSSION

It is no surprise that there are differences among the 3 crane species/subspecies kept at Patuxent. The results point to the uniqueness of each group and to the value of identifying normal blood values for each species or subspecies.

Historically, hematology and serum chemistry values seen in cranes have changed, thus necessitating establishing new standards. Total protein, globulin, albumin/globulin ratio, and uric acid values were all significantly different from the historic normal values reported from the 1970's by Gee et al. (1981). Total protein values are subject to variance depending on the method used. Total protein obtained from plasma can be up to 1.0 g/l higher than that obtained from serum due to the presence of fibrinogen (Lumeij 1987). Furthermore, there is a difference in methodology with readings of serum or plasma taken using a refractometer being consistently higher than those obtained by the biuret method (Lumeij 1987). Indeed, today we refer to the refractometer method as giving us a reading in total solids, not total protein. Even when using the refractometer method to measure serum or plasma total solids, it is now known that it is important to use a temperature compensated refractometer (Fudge 1997).

The uric acid levels in our study were significantly lower in all 3 crane groups as compared to the historic values from Gee et al. (1981). High values for uric acid may be a result of the technique used to obtain the sample or may represent kidney disease (Fudge 1997). In addition, uric acid is synthesized in the kidney (Lumeij 1987). The historic blood samples were collected in the period before disseminated visceral coccidiosis (DVC) was recognized as an important disease in the crane population at Patuxent. Focal granulomatous nodules caused by the causative organisms (Eimeria gruis or E. reichenowi) form in a number of tissues including the heart, liver, spleen, and kidney (Carpenter 1980, 1984). This may also account for the significant difference seen in greater sandhill cranes between the historic data and current values. Liver or heart damage can lead to elevated levels of lactic dehydrogenase, and these organs are both affected by DVC. For the past 2 decades DVC has been controlled at Patuxent by using coccidiostatic medicines as feed additives (Olsen and Carpenter 1997).

Table 4. A comparison of blood parameters for whoopingcranes between current and historic values at USGS PatuxentWildlife Research Center using t-tests to compare means.

Variable	Curr Valı	rent 1es	Histo Valu	ť	
	Mean	n	Mean	n	
Hematocrit (%)	40.0	29	42.0	15	1.84°
Glucose (mg/dl)	236	29	232	16	0.45
Total protein (g/dl)	3.31	29	3.80	16	4.06°
Albumin (g/dl)	1.55	29	1.50	16	-1.06
Alkaline phosphatase (IU/l)	170.4	29	46.0	16	6.17°
Lactic dehydrogenase (IU/I)	348.5	29	440.0	16	1.59
Calcium (mg/dl)	8.99	29	9.10	16	0.61
Phosphorus (mg/dl)	3.44	29	2.80	16	1.59
Globulin (g/dl)	1.76	29	2.30	16	-9.55°
Albumin/globulin	0.87	29	0.65	16	7.64°
Uric acid (mg/dl)	5.95	29	8.10	16	2.98°

* Historic values from Gee et al. (1981).

^b t = student's t value.

Significance was assumed if P < 0.05.

The results reported in this study should provide a new benchmark to measure the health of the captive crane populations at Patuxent. The results should also be applicable to other institutions keeping these crane species under similar conditions. Because of the large size of the crane flock at Patuxent, we were able to obtain large sample sizes for this study. The methodologies used are well established and standardized at this point in time. Of course, we make these statements on the assumption that the crane population at Patuxent is healthy and that there are no serious unknown diseases at this time. Some of the indicators of the health of the crane population at Patuxent are the record number of eggs and young produced each year and the ages of some of the cranes in the population, with a few of the cranes being 35 years old at the time of the study.

In summary, both a healthier crane population and improvements in sampling techniques have apparently altered the hematological and serum chemistry norms in the Patuxent colony. New standards (as presented herein) need to be used and updated as needed.

	0 (22)		1 (4)		2-5 (5)		6–15 (15)		>15 (4)		
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
Heterophils (%)	72.3	11.1	49.3	15.9	62.8	4.8	63.9	5.2	64.5	5.4	0.2481
Lymphocytes (%)	24.3	10.6	46.8	16.5	36.2	4.8	34.7	5.2	33.8	5.1	0.1042
Monocytes (%)	1.5	1.3	2.5	1.3	0.4	0.5	0.2	0.4	0.8	1.0	0.0049
Eosinophils (%)	1.7	1.2	1.5	1.9	0.6	0.5	1.2	0.6	1.0	0	0.1553
H/E count (No./mm ³)	9400	3690	5620	1540	7380	3550	7680	3485	6960	1585	0.2768
White blood cells (No./mm ³)	12600	4310	11185	1445	11485	4890	11790	3290	10560	1660	0.5467
Hematocrit (%)	33.0	7.0	44.0	3.5	38.4	1.9	39.1	0.3	40.8	6.1	0.0058
Total solids (g/dl)	3.6	0.5	4.2	0.6	4.0	0.3	4.1	0.4	4.4	0.2	0.0011
Glucose (mg/dl)	250	23	257	9	249	17	256	30	256	34	0.4792
Total protein (g/dl)	3.0	0.4	3.2	0.6	2.9	0.5	3.1	0.3	3.4	0.2	0.0577
Albumin (g/dl)	1.3	0.1	1.5	0.4	1.3	0.1	1.4	0.1	1.6	0.1	0.0630
Alkaline phosphatase (IU/l)	759	222	459	513	223	219	231	102	207	95	0.0001
Aspartase aminotransferase (IU/I)	228	48	232	53	145	26	166	38	160	14	0.0006
Lactic dehydrogenase (IU/l)	861	247			259	65	290	106	271	115	0.0001
Calcium (mg/dl)	10.7	0.7	10.0	0.5	9.2	0.2	9.2	0.5	9.4	0.4	0.0001
Phosphorus (mg/dl)	4.4	1.7	4.9	0.9	4.2	2.7	3.6	1.0	3.8	0.2	0.1459
Albumin/globulin	0.84	0.27	0.90	0.18	0.88	0.19	0.83	0.12	0.88	0.15	0.8053
Globulin (g/dl)	1.6	0.3	1.7	0.3	1.5	0.4	1.7	0.2	1.8	0.2	0.1397
Creatine kinase (IU/I)	3520	2243	356	174	384	70	368	115	380	133	0.0001
Uric acid (mg/dl)	11.1	3.3	9.6	2.8	10.2	1.5	7.4	2.4	7.9	2.1	0.0025

Table 5. Means and standard deviations of blood parameters for captive Florida sandhill cranes at USGS Patuxent Wildlife Research Center by age group (young-of-the-year [0], 1-year-old, 2-5 years-old, 6-15-years-old, and >15-years-old).*

* Numbers in parenthesis are sample sizes.

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Variable	0	(4)	1(1)		2-5	(9)	6–15	(12)	>15	(7)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Р
Heterophils (%)	64.8	7.6	65.0	0	63.6	5.1	62.5	5.5	67.1	3.0	0.1764
Lymphocytes (%)	34.0	7.4	33.0	0	35.2	5.0	35.6	5.2	30.7	2.9	0.1453
Monocytes (%)	0.3	0.5	0	0	0.4	0.5	0.7	0.5	0.7	0.8	0.2481
Eosinophils (%)	1.0	0	2.0	0	0.8	0.4	1.3	1.5	1.3	1.4	0.6022
H/E count (No./mm ³)	6340	1320	1985	0	6000	2060	6290	1580	7460	1850	0.0651
White blood cells (No./mm ³)	9575	1010	2961	0	9385	3390	9840	2280	10860	2410	0.1383
Hematocrit (%)	41.5	0.6	42.0	0	40.9	2.8	37.7	3.6	36.6	3.9	0.0018
Total solids (g/dl)	3.5	0.1	3.8	0	4.3	0.4	4.2	0.5	3.9	0.5	0.8371
Glucose (mg/dl)	249	12	295	0	221	39	250	29	272	20	0.0367
Total protein (g/dl)	2.4	0.1	2.8	0	3.1	0.2	3.1	0.2	3.1	0.5	0.1800
Albumin (g/dl)	1.3	0.1	1.4	0	1.5	0.1	1.5	0.1	1.4	0.1	0.4636
Alkaline phosphatase (IU/l)	558	97	242	0	377	116	319	248	364	162	0.6611
Aspartase aminotransferase (IU/I)	223	19	150	0	199	30	202	49	168	21	0.0106
Lactic dehydrogenase (IU/l)	364	93	257	0	207	32	222	83	194	42	0.0635
Calcium (mg/dl)	9.7	0.4	9.1	0	9.5	1.0	9.0	0.6	8.8	0.5	0.0558
Phosphorus (mg/dl)	5.1	1.4	2.8	0	4.5	2.4	3.0	0.7	2.9	1.0	0.0341
Albumin/globulin	1.15	0.06	0.5	0	0.96	0.15	0.91	0.12	0.84	0.20	0.0069
Globulin (g/dl)	1.1	0.1	1.4	0	1.6	0.2	1.6	0.2	1.7	0.4	0.0125
Creatine kinase (IU/1)	336	0	550	0	343	167	260	97	207	31	0.0381
Uric acid (mg/dl)	5.3	1.5	7.1	0	8.2	3.6	5.2	1.5	5.3	1.8	0.1511

Table 6. Means and standard deviations of blood parameters for captive greater sandhill cranes at USGS Patuxent Wildlife Research Center by age group (young-of-the-year [0], 1-year-old, 2-5-years-old, 6-15-years-old, and >16-years-old).^a

* Numbers in parenthesis are sample sizes.

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	0(13)		1 (3)		2-5	2-5(8)		6-15 (15)		>15(1)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
Heterophils (%)	61.5	8.3	65.7	1.5	67.0	7.4	63.3	16.7	67.0	0	0.3762
Lymphocytes (%)	33.7	7.8	33.0	1.7	29.5	9.3	32.5	11.7	31.0	0	0.5568
Monocytes (%)	1.7	2.3	0.3	0.6	1.0	1.4	1.1	1.7	7	0	0.2571
Eosinophils (%)	2.6	3.0	2.4	1.0	3.0	3.0	2.9	4.6	2.0	0	0.9321
H/E count (No./mm ³)	8270	2685	8115	735	11690	2030	9720	2285	7520	0	0.1771
White blood bells (No./mm ³)	12950	4340	12165	910	16910	2480	15195	4435	10900	0	0.2953
Hematocrit (%)	41.8	3.2	42.0	2.0	38.4	2.9	37.7	3.2	42.0	0	0.0107
Total solids (g/dl)	4.2	0.4	3.9	0.6	4.3	0.5	4.2	0.3	3.5	0	0.8189
Glucose (mg/dl)	248	20	240	58	241	33	236	28	211	0	0.1769
Total protein (g/dl)	3.2	0.4	3.4	0.2	3.4	0.5	3.5	0.3	3.6	0	0.4665
Albumin (g/dl)	1.5	0.1	1.5	0.2	1.5	0.2	1.6	0.1	1.7	0	0.7706
Alkaline phosphatase (IU/l)	238	92	183	15	125	38	133	43	105	0	0.0012
Aspartase aminotransferase (IU/I)	331	80	266	78	282	67	277	52	271	0	0.1476
Lactic dehydrogenase (IU/l)	334	89	354	77	437	175	345	89	612	0	0.4128
Calcium (mg/dl)	9.3	0.4	8.9	0.2	9.3	0.8	9.0	0.5	9.4	0	0.3995
Phosphorus (mg/dl)	3.7	1.1	3.0	1.2	3.7	1.3	3.4	1.7	3.0	0	0.6263
Albumin/globulin	0.96	0.28	0.83	0.06	0.83	0.09	0.87	0.10	0.90	0	0.4294
Globulin (g/dl)	1.7	0.3	1.83	0.06	1.9	0.3	1.8	0.2	1.9	0	0.3295
Creatine kinase (IU/l)	585	266	359	180	285	129	227	138	844	0	0.0553
Uric acid (mg/dl)	6.3	3.0	6.0	2.0	53	1.3	5.0	1.6	10.1	0	0.8017

Table 7. Means and standard deviations of blood parameters for captive whooping cranes at USGS Patuxent Wildlife Research Center by age group (young-of-the-year [0], 1-year-old, 2-5-years-old, 6-15-years-old, and 16-years-old).^a

* Numbers in parenthesis are sample sizes.

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