

SERUM CHEMISTRY VALUES AND ISOLATED MICROBIAL ORGANISMS FOR SWALLOW-TAILED KITE, *ELANOIDES FORFICATUS*, NESTLINGS IN SOUTHEASTERN UNITED STATES

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ABSTRACT: *The analysis of serum chemistries is becoming a vital diagnostic tool for assessing the health of free-ranging birds of prey. The serum chemistries of 21 free-ranging swallow-tailed kites, *Elanoides forficatus*, nestlings were measured in 1995 and 2003 in several regions of Florida, Georgia and South Carolina. One hematological value (PCV) and twenty-four serum values were measured to establish normal baseline parameters for the kites. Microbial health assessment applications in wildlife monitoring are in the infancy stage. Microbial organisms were identified from cloacal and choanal swabs. *Escherichia coli* (88%), *Enterobacter cloacae* and *Klebsiella pneumoniae* (44%) and *Proteus mirabilis* (43%) were the predominant microbial organisms.*

Key Words: Swallow-tailed Kites, hematology, serum chemistry, microbial organisms, health assessments

CLINICAL studies of serum chemistries of free-living birds of prey are beginning to permeate the literature (Bowerman et al., 2000; Balbontin and Ferrer, 2002; Casado et al., 2002; Mealey et al., 2004). Serum chemistries can play an important and valuable role in the formulation of differential diagnosis and prognosis in captive avian medicine. These chemistry values are the result of metabolic and other physiological processes in the birds (Harrison and Harrison, 1986). The measurements of several chemical compounds in the blood can be used in the diagnosis of the health status of analyzed individuals (Harrison and Harrison, 1986).

Establishing normal baseline values for the serum chemistries of free ranging birds of prey will be important for future comparisons seeking to understand population trends and health (Tatum et al., 2000). Discerning the significance of serum chemistry values must be done with prudence since age, sex, nutritional status and environmental conditions, circadian rhythms and plasma and serum storage methods may influence these values (Ferrer, 1993; Bustamante and Travianí, 1993; Boal et al., 1998). Until a more complete database has been established for avian serum chemistries and their application as a monitoring tool in free-ranging raptors,

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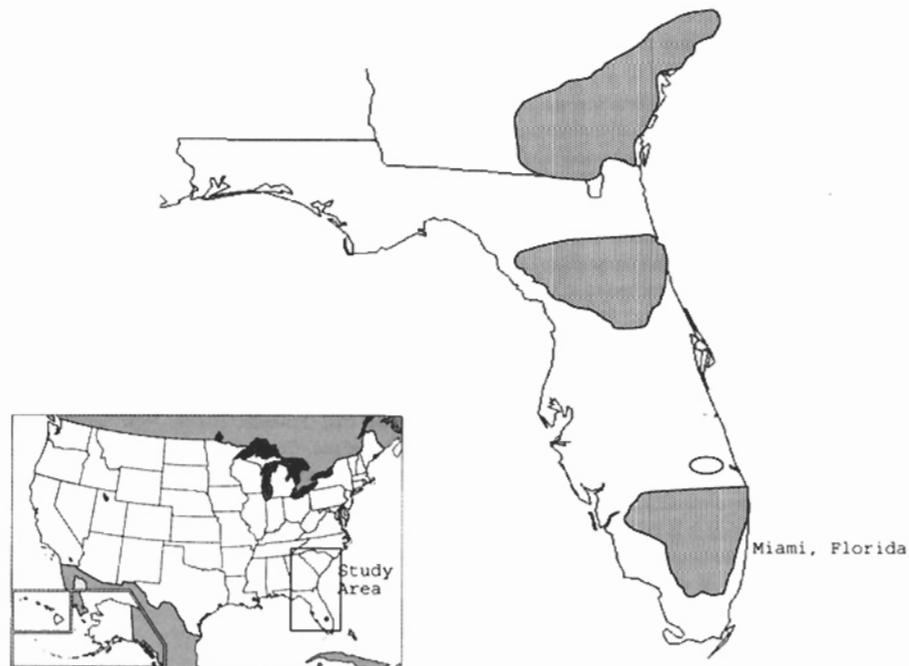


FIG. 1. The study sites (shaded) where nestling swallow-tailed kites were sampled for the serum chemistry and microbial study in 1995 and 2003 were located in Florida, Georgia and South Carolina, USA.

these values should be used in combination with complementary ecological data when making population assessments (Newman et al., 1997).

Hematological analyses provide valuable information concerning the health status of an individual animal. Packed cell volume (PCV) is a good indicator of red blood cell mass (Howard and Matsumoto, 1977) but age, sex (Boal et al., 1998), molting and reproductive cycles, migration, dehydration and diseases can affect avian PCV's (Carpenter, 1975; Heidenreich, 1997; Morishita et al., 1998).

The objective of this study was to determine selected clinicopathologic parameters for nestlings of free-ranging swallow-tailed kites, *Elanoides forficatus*. Since knowledge of the basic physiology of these animals is limited, this protocol will contribute to establishing "normal" baseline hematological and serum values for swallow-tailed kite (STKI) nestlings in Southeastern United States.

MATERIALS AND METHODS—Study site—Samples were collected in three broad areas: southern Florida (mainly Everglades National Park and Big Cypress National Preserve); northern Florida (industrial timberlands); and the coastal plain of Georgia (industrial and privately-owned timberlands).

Ageing and sexing—Nests containing STKI nestlings from 35 to 45 days old were visited from 1 May through 15 July 1995 and 2003. Nestling development was monitored chronologically or through feather formation by way of repeated nest visits (Bortolotti, 1984). Sexing of the nestlings was conducted from the blood samples using DNA analysis provided by K. Norris-Canada (1995) and Avian Biotech

International, 1336 Timberlane Road, Tallahassee, Florida 32312-1766 (2003). Most of the nests were surveyed no more than three times. Each nest was visited only once for blood sampling.

Blood samples—Blood sampling was conducted as often as possible during mornings, with only a few samples taken later in the day or evening. Circadian rhythms were considered prior to sampling, but the window within the sampling period was extremely narrow due to seasonal road inaccessibility and other logistical constraints, resulting in opportunistic sampling. Nestlings were removed from the nests by the investigators and hooded with a traditional falconry hood manufactured by Northwoods, Inc. P.O. Box 874, Rainier, WA, 98576. Blood was extracted from the brachial vein (Cooper, 1975; Hoysak and Weatherhead, 1991). The area surrounding the vein was cleaned with 70% isopropyl alcohol and a sterile 22, 23 or 25-gauge needle attached to a 3-ml syringe (Becton Dickinson & Co.), was used to extract 1–3 ml of blood from each nestling. All blood extraction sites had pressure applied and were observed for approximately 5 minutes post sampling to insure proper clotting prior to placing the nestling back into the nest (Hoysak and Weatherhead, 1991). We returned to each nest approximately 7–10 days post sampling to re-evaluate the nestlings' condition. Blood samples were placed in small 1-ml red top plastic microtainer tubes with a serum separator (Becton Dickinson Co., Franklin Lakes, New Jersey) and heparinized hematocrit tubes, (Jorgensen Laboratories, Inc., Loveland, Colorado), and allowed to clot for 15 minutes prior to centrifugation. Samples were spun for approximately 20 minutes with a portable Mobilespin centrifuge manufactured by Vulcan Technologies, MO, with a relative centrifugal force of 1100× g. Centrifuging was complete when the serum separator distinctly walled off the serum from the red blood cells. The serum was transferred with a pipette to another blood tube to prevent hemolysis. Field samples were placed in an ice cooler and later frozen. Samples were stored at -16.1°C and analyzed within 30 days.

Serum samples—Kite sample sizes varied for each serum chemistry test depending on the amount of serum available. Some results were above or below the analyzer's range, which resulted in smaller sample sizes. One hematological, packed cell volume (PCV), and nineteen serum chemistries were determined including: total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), lactate dehydrogenase (LDH), creatine kinase (CK), uric acid (URIC), calcium (Ca^{2+}), phosphorus (PHOS), glucose (GLU), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (CRSC), sodium (Na^+), potassium (K^+), chloride (Cl^-), carbon dioxide (CO_2) and cholinesterase (Che). Blood samples were analyzed on a Kodak Ektachem DT II System (Johnson & Johnson, Rochester, New York). Analysis and methodology followed the protocol in the Johnson & Johnson Ektachem DT II System manual. Ektachem controls were run once a week to assure quality analysis control with all samples. Mean values and standard deviations were determined using JMP SAS statistical discovery software (JMP SAS, 2003).

Microbial organisms—Microbial analyses were also performed to determine the presence and frequency of microbial species. The utility of routine bacteriological culture in captive management has become increasingly important in recent years; however, the monitoring of wild populations is still in its infancy (Harwood et al., 1999). We utilized sterile culture swabs and media in the field to collect a sample from the cloaca and choana of each individual. A culture was made by introducing the sterile swab into the cloaca and choana of the individual, withdrawing the swab, and placing it into the media tube. The applicator was placed in a growth medium and transported to the laboratory. Exceptional caution was required during the culture acquisition to insure that neither instruments, nor external surfaces on the birds or the environment itself contaminated the sample. Each cloacal culture resulted in the isolation of one to several microbial organisms. Cultures were sent to Micrim Laboratories, 800 N.E. 62 Street, Suite 202, Ft. Lauderdale, Florida 33334, for analysis within 48 hours of collection.

RESULTS AND DISCUSSION—The results for serum chemistry and PCV values for the STKI nestlings are shown in Table 1. The range was variable for several chemistries including LDH, CK, ALKP, AST and URIC. Some results were above

TABLE 1. Hematological and serum chemistry values for 21 swallow-tailed kite nestlings in Southeastern United States.

Measure	N	Mean	SD	Range
PCV (%)	9	33.78	5.23	26.0–40.0
WBC (%)	9	1.1	0.33	1.0–2.0
GLU (mg/dl)	19	279.1	41.01	188.0–397.0
PHOS (mg/dl)	16	5.5	2	1.0–8.7
TP (g/dl)	21	4.34	1.47	2.2–10.0
URIC (mg/dl)	17	14.38	6.07	8.8–32.0
ALKP (U/L)	4	196.75	61.17	150.0–285.0
AST (U/L)	18	184.33	107.37	54.0–491.0
Ca (mg/dl)	20	8.63	1.37	5.2–10.5
CHE (U/ml)	6	0.8	0.32	0.26–1.19
CK (U/L)	16	3084.44	2413.44	322.0–8776.0
LDH (U/L)	12	5601.5	3314.73	1726.0–11736.0
CRSC (mg/dl)	4	0.15	0.05	0.10–0.20
BUN (mg/dl)	10	3.7	1.25	2.0–5.0
CREA (mg/dl)	10	0.28	0.13	0.10–0.50
BUN/CREA	9	15.55	9.41	10.0–40.0
Na+ (mmol/L)	15	144.73	12.5	107.0–155.0
CL- (mmol/L)	3	117.33	21.07	93.0–130.0
K+ (mmol/L)	13	3.28	1.32	1.0–5.3
CO ₂ (mmol/L)	12	21.08	4.35	17.0–33.0
Amylase	9	1510.56	782.98	180.0–2343.0
Lipase	10	52.8	77.49	15.0–272.0
GGT	10	12.8	6.33	8.0–24.0
Cholesterol	10	284.9	75.92	168.0–403.0
Triglyceride	10	284.9	17.05	32.0–90.0

or below range measurable by the Kodak platform's range, which resulted in lower sample sizes. We successfully completed 18 cloacal and choanal cultures from which twenty different organisms were isolated (Table 2).

Currently the interpretation of avian biochemistries and the impact of microbial organisms are limited due to the lack of controlled studies and available references (Ferrer, 1993). Raptor biologists and wildlife veterinarians must also be aware that values among adults, juveniles and nestlings may vary significantly (Boal et al., 1998). These data were used to establish baseline parameters of free-ranging STKI nestlings in the southeastern United States. Even though raptor nestling serum values are not well documented in the literature, the results of this study appear to fall generally within the reported ranges for other free-ranging raptor nestlings (Boal et al., 1998; Dobado-Berrios et al., 1998; Stein et al., 1998; Bowerman et al., 2000; Balbontin and Ferrer, 2002; Casado et al., 2002).

The PCV values of free-ranging raptors are quite variable (Balasch et al., 1976; Hunter and Powers, 1980). Variation may be a consequence of age, sex, migration status, and reproductive status (Boal et al.; 1998, Stein et al., 1998). Our PCV mean values of 33.78% (Table 1) for STKI nestlings are within the range (31–38%) reported for free-ranging nestling eagles in Florida Bay (Mealey et al., 2004). In contrast, results for PCV mean values for free-ranging nestling Cooper's Hawks

TABLE 2. The microbial results of 18 choanal and cloacal cultures of swallow-tailed kites taken immediately upon capture. Unshared microbiota are designated by a dash in the corresponding column.

Microbial Organism	Choanas		Cloacas	
	No. Isolated	% of Isolations	No. Isolated	% of Isolations
<i>Escherichia coli</i>	14	88	18	100
<i>Enterobacter cloacae</i>	9	56	3	21
<i>Pseudomonas aeruginosa</i>	1	6	—	—
<i>Staphylococcus aureus</i>	2	13	1	7
<i>Enterococcus sp.</i>	3	19	4	25
<i>Proteus mirabilis</i>	2	13	6	43
<i>Bacillus sp.</i>	—	—	1	7
<i>Klebsiella pneumoniae</i>	7	44	7	44
<i>Salmonella sp.</i>	—	—	1	7
<i>Edwardsiella tarda</i>	—	—	1	7
<i>Acinetobacter pneumoniae</i>	1	6	—	—
<i>Acinetobacter baumannii</i>	2	13	1	7
<i>Citrobacter freundii</i>	2	13	2	14
<i>Morganella morganni</i>	—	—	2	14
<i>Pseudomonas flourescens</i>	2	13	2	14
<i>Acinetobacter hemolyticus</i>	1	6	—	—
<i>Staphylococcus maltophilia</i>	1	6	—	—
<i>Aeromonas hydrophila</i>	1	6	—	—
<i>Serratia marcescens</i>	1	6	—	—
Budding yeast	5	31	3	21

(*Accipiter cooperii*) was 42.2 % for females and 38.7% for males (Boal et al., 1998). Seabirds PCV mean values ranged from 40–57.9%, which are much higher and may be attributed to the marine environment (Wanless et al., 1997).

Most normal avian values for total protein range between 3.0 and 5.0 g/dl (Heidenreich, 1997). Values that fall below 2.5 g/dl may reflect parasitism, stress or starvation. Values greater than 5 g/dl may indicate dehydration, shock or infection (Harrison and Harrison, 1986). Plasma protein levels were found to vary in female American Kestrels during prelaying and incubation (Dawson and Bortolotti, 1997). Total protein values in this study ranged from 2.2–10 g/dl with a higher variation than those in Michigan (Bowerman et al., 2000). The mean values for total protein 4.234 g/dl were similar to Michigan Bald Eagles at 3.4 g/dl but lower than the 4.7 g/dl found in Bald Eagles at Chippewa National Forest (Redig et al., 1983).

Uric mean and range values for STKI nestlings were similar to Florida Bay Bald Eagle populations of 13.46 mg/dl but higher than the Bonelli's Eagle (*Hieraetus fasciatus*) nestling mean values of 7.77 mg/dl (684.2 $\mu\text{mol L}^{-1}$) for males and 9.4 mg/dl (827 $\mu\text{mol L}^{-1}$) for females (Balbontin and Ferrer, 2002; Mealey et al., 2004). Brown Booby levels were greater than these raptors with values of 20.5 mg/dl for females and 16.7 mg/dl for males (Work, 1999).

Mean GLU levels in STKI nestlings of 279.1 mg/dl falls within the normal range of 200 and 500 mg/dl found among captive birds of various species. Electrolyte results for Na^+ , K^+ and Cl^- in Florida Bay were within the ranges of other raptor nestling studies (Redig et al., 1983; Bowerman et al., 2000). There were

variations (Table 1) when compared with the Bonelli's Eagle (Balbontin and Ferrer, 2002) and below the values of several European raptors (Polo et al., 1992; Jenkins, 1994; Stein et al., 1998).

High variation of CK and LDH levels in this study may be due to the handling and physiology of nestling STKI and fall within the range in free-living Bonelli's Eagle, Booted Eagle (*Hieraaetus pennatus*), the Spanish Imperial Eagle (*Aquila adalberti*) and the Bald Eagle (Polo et al., 1992; Balbontin and Ferrer, 2002; Casado et al., 2002; Mealey et al., 2004).

CHE levels < 0.9 u/ml are considered depressed and likely due to intoxications (Porter, 1993; Heatley and Jowett, 2000). CHE mean value for the STKI nestlings was 0.8 u/ml and within the levels considered depressed. STKI sample size was small and difficult to analyze as a consequence. These nestlings all successfully fledged in 1995. In 1997, similar findings were obtained in Florida Bay, where two eaglets, 35 days old, were found to have depressed levels of CHE. Both eaglets successfully fledged. These eaglets may have been exposed to organophosphates after consuming avian prey delivered by their parents (Mealey et al., 2004). In contrast, Osprey from Florida Bay had CHE levels above 1.0 u/ml (Mealey, unpublished data). These interpretations should be made with caution as there is controversy in using CHE as an indicator for exposure to an organophosphate pesticide due to the variations in CHE levels over short periods of time (Mineau, 1998).

The presence of microbial organisms does not signify a compromising infection since a degree of symbiosis occurs. Many of these organisms assist in the avian digestive process and in the absorption of nutrients. Potential pathogens, (e.g. *Salmonella spp.*), may be acquired through the ingesting of selected prey and perhaps eliminated over a period of time. Twenty different microbes were isolated from the swabs. Our microbial cultures provide the first measures of these microfauna for free ranging swallow-tailed kites nestlings. The results are not unexpected in that most current clinical studies recognize susceptibility across taxa to a given microbe (Jacobson, 1999).

Evaluation of serum chemistries and microbial fauna offers wildlife biologists and agency managers an additional way of monitoring the health of free-ranging bird of prey populations. If the opportunity exists to gather such information about a listed raptor population, then every effort should be made to achieve and enhance the existing pool of knowledge concerning a species (Ferrer, 1994). As with every technique, precautions should be taken to avoid any potential injury or undue stress to the animals.

Currently, data interpretation from serum chemistries and microbial infections are subject to debate because many variables are likely to affect a particular enzyme or value. As standardized protocols are established and long-term studies on the baseline values of a particular species conducted, the interpretation of the results will become less contentious. Areas in specific need of attention and further research with free-living raptors are the effects of (1) capture and handling and how this stress may affect results; (2) age, sex and season on serum values; and (3) nutrition on specific serum value indicators. Yet, the refinement of this analytical tool requires that values from wild animal populations be assembled and synthesized to generate

the basis for future refinements of interpretation. Analyzing serum chemistries is in its infancy stage for free-ranging birds of prey. As further research is conducted, the information gathered will aid in the applications of serum chemistry analysis as a tool for the management of raptor populations. This should not act as a deterrent to future investigators, as without data collected and published from populations over time the enhancement of our understanding of these endangered raptor populations will not be achieved.

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