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Saving Lives: Critical Blood Analytes for Rehabilitation of Coastal Birds

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ABSTRACT 300224:

Coastal bird species are often affected by oil spills and can suffer damage to the kidneys, adrenal glands, salt glands, and gastrointestinal tract. Although hypothermia is often touted as the proximate cause of avian death in oil spill events, birds can ultimately succumb to these petroleum product-related injuries based on blood electrolyte and acid-base abnormalities caused by multiple organ dysfunction. However detection and treatment of these analyte abnormalities remains challenging because we know little of the nature of analytes in the healthy water bird. Blood samples were obtained from free living and rehabilitated healthy Mottled ducks (*Anas fulvigula)*, Black-bellied whistling ducks *(Dendrocygna autumnalis),* Yellow-Crowned Night Herons *(Nyctanassa violacea)* and Brown Pelicans *(Pelecanus occidentalis)* from 2008-2012. Blood gas, electrolyte, and select biochemical and hematological parameters were determined in the field with a patient side analyzer while complete blood counts, packed cell volumes and osmolalities were determined by standard laboratory methods. Reference intervals and 95% confidence intervals were determined and the effect of age, sex, body condition, time and date of sampling were also assessed for all analytes. Species, lifestyle, environment, and diet all appear to have an effect on "normal" electrolyte and acid base analytes in coastal birds. An increased plasticity of and tolerance to change in blood analytes may be a normal finding in these species which may facilitate care in the response setting. The reference intervals of apparently healthy coastal birds -differed from mammalian values in some instances, however these intervals will assist in the appropriate diagnosis and treatment of these species in rehabilitation and oil spill response. These values can be used to assist biologists, rehabilitators and veterinarians in assessment, management and treatment of avian species in the event of environmental disturbance, such as oil spill, draught, or tropical storm as well as to assess overall ecosystem health via coastal birds as indicator species.

INTRODUCTION:

Physiologic reference intervals are an important tool used to guide individual clinical diagnoses and therapy and assessment of population health. This is particularly true when individuals or species are suffering critical illness or injury such as petroleum product exposure. However, valid reference intervals for free-living avian species are rare, based on the difficulty in capturing sufficient numbers of healthy individuals in order to establish normal data ranges. Reference intervals for several species within an ecosystem could provide a useful measure of ecosystem health.

Current methods for evaluating ventilation/perfusion and damage to organ systems using plasma enzymes fail in birds, as they are neither sensitive nor specific for organ function. Parameters for venous blood gases, hematology, and lactate in apparently healthy avian species will facilitate identification of pathophysiologic processes and serve to guide prognosis and therapy, especially during environmental disturbances along the Gulf Coast such as an oil spill, drought, or tropical storm. Having clinicopathologic parameters for coastal birds allows for assessment of the health of an individual species, as well as an estimation of the well-being of an entire ecosystem.

Fluid therapy is the cornerstone of restoring avian health in an oil spill response. However little is known of the proper constituents of avian extracellular fluid. Knowledge of electrolytes, plasma osmolality, venous blood gases and plasma enzymes guide emergency treatment in other species such as humans and dogs. But these analytes remain poorly investigated in birds, especially the bird species commonly affected by oil spill events along the Gulf Coast. Thus best achievable diagnosis and care is currently based solely on information obtained from two tests: determination of packed cell volume and total solids concentration.

While these tests are relatively fast and inexpensive, they give limited information for diagnosis and treatment of birds. Multiple analytes in birds can now be determined from as little as 0.2 mls of blood within 2 minutes using point of care analyzers. Reference intervals should be determined for these analytes. Veterinarians and rehabilitators are hampered by lack of references ranges to determine blood abnormalities during oil spill response as well as a lack of knowledge of appropriate fluids for treatment. This study proposes to close that gap in knowledge for common species along the Gulf Coast which are likely to be affected by petroleum.

Osmolality is a measure of soluble particles found in the plasma without regard to size, weight, or charge of these particles. In biological systems, the relative osmolality of the intracellular and extracellular spaces determines the fluid volume in each compartment and therefore, this value is closely regulated. Knowledge of the plasma osmolality of a particular species is of paramount importance when formulating fluid treatment for a patient as abnormalities in this system can quickly lead to life-threatening fluid and electrolyte imbalances. The osmolality of most mammals is approximately 300 mOsm/L, thus most commercially available fluids for the treatment of shock and dehydration are near this number. Recent studies in a healthy parrot species have shown that these birds have a much higher osmolality (326 mOsm/L)(Acierno et al. 2009). Determination of the plasma osmolality of healthy seabirds will provide an important guide for fluid therapy in birds affected by oil. Fluids can be tailored to reflect the appropriate osmolality prior to administration. Appropriate fluid therapy will allow for a greater recovery and release rate from oil spill events for avian species.

Electrolyte abnormalities are expected in birds affected by oil based on known damaging effects of petroleum on multiple organs. Electrolyte and acid-base balance of the blood is closely regulated by the body and has direct impact on heart and other vital organ function. Organs known to be damaged by oil include the kidneys, gastrointestinal tract, adrenal glands and salt glands (Fry and Lowenstein 1985; Tseng 1999). These organs work in synchrony to maintain proper electrolyte balance in the coastal environment. Damage to these organs can result in severe life threatening electrolyte abnormalities, which likely occur in birds affected by oil. Exposure to petroleum products can also result in lung damage and lysis of red blood cells (Leighton 1983, Leighton 1985). These abnormalities likely also result in venous blood gas abnormalities, which could also be life threatening. Determination of these abnormalities is key in determining appropriate fluid and other supportive therapies in birds affected by oil.

However, these expected electrolyte and blood gas abnormalities have not been systematically investigated in avian species affected by oil. Thus most recommendations for avian fluid therapy are assumptions based on findings in mammalian models (Hernandez and Aguilar 1994; Steinhort 1999; Abou-Madi and Kollias 1992). This is likely based on a number of factors including a lack of reference intervals for Gulf Coast bird species, the large blood sample volume necessary for many analyzers, and the time necessary for shipment and receipt of results from reference laboratories. However, new point of care analyzers are portable, require small blood samples, give results in two minutes, and can be used in birds (Paula et al. 2008; Steinmetz at al 2007; Acierno et al. 2008).

Few reference intervals for osmolality, venous blood gases or electrolytes are available for wild or nondomestic animals, and seabirds affected by oil spill events are no exception (Zaias et al. 2000; Stoskopf et al. 2010). Standard practice for determining analyte reference intervals via appropriate statistical models includes sampling a minimum of 60 apparently healthy animals (Stockham and Scott 2008). Reference intervals for these analytes will improve health assessment and guide fluid therapy in birds. The findings will further the goals of improving survival and release rate of birds affected by petroleum exposure along the Gulf Coast. The purpose of this prospective study was to create reference intervals for venous blood gases, electrolytes, and hematology using the iSTAT-1® point of care analyzer. We also analyzed the effect of gender, age, capture year, capture type and time of day on these analytes when possible per species.

Avian species were selected based on stable populations, individuals large enough to safely allow adequate blood sample volume to be collected and subsequently allow immediate and safe release of the individual bird, known susceptibility to petroleum exposure and likelihood of presentation for and survival of either trapping or rehabilitation (Deepwater 2011). Herons have a medium susceptibility while the ducks, pelicans and gannets have a high probability of petroleum exposure (Rocke 2011). The Mottled duck is a species of concern along the Gulf Coast and was also being studied, banded and trapped by Texas Parks &Wildlife Department. These species represent a variety of lifestyle niches, dietary preferences, hunting styles, and habitat preferences (Table 1). Thus this range of species should represent and allow data extrapolation, to other similar species affected by petroleum exposure until further data is obtained. The determined reference intervals may be used by health care providers during oil spill response. During oil spill response, these intervals may be used to improve for health assessment in birds affected by oil. Fluid composition can then be tailored to better meet avian physiological needs, at the population or herd level, during oil spill response. Results will also allow for a more appropriate fluid therapy in birds, based on electrolyte abnormalities in the patient, increasing the standard of care and the number of birds returned to health and their natural environment.

Table 1. Texas Gulf Coast Bird Species prioritized for Reference Interval creation

Common Name	Species Name	IUCN Status	Habitat	Diet	Foraging Style
Yellow-crowned night heron	*Nyctanassa violacea*	Least concern	Near Shore, migratory & permanent resident	Fish, frogs, crustaceans	Wading, stalking
Brown Pelican	*Pelicanus occidentalis carolinensis*	Least concern, Endangered in Texas	Near Shore, coastally migratory	Fish	Plunge diving
Mottled Duck	*Anas fulvigula maculosa*	Near threatened	Near Shore, mostly non migratory	Plants, mollusks, insects	Dabbling, grazing
Black Bellied Whistling Duck	*Dendrocygna autumnalis*	Least concern	Near Shore, nonmigratory	Plants, arthropods, invertebrates	Wading

MATERIALS AND METHODS:

Sample analysis

We sampled an appropriate number of prioritized coast bird species which were healthy to appropriately determine osmolality, electrolyte, and venous blood gas reference intervals for representative Gulf Coast species of birds. From 12-129 individuals from each of the prioritized species were sampled in collaboration with licensed rehabilitators and the Texas Parks and Wildlife Department. For each species, 1.5 mL of blood was collected via jugular venipuncture with needle and syringe. Samples were placed into green-top lithium heparin tubes (Sarstedt Inc, Newton, NC, USA) to allow for complete blood count, blood gases, biochemical and electrolyte analysis. Analysis for blood gases and electrolytes was performed at patient side in the field within 3-5 minutes of sample collection using the Abaxis iSTAT-1® analyzer(Abbott Laboratories. Abbott Park, IL USA, Abbott Point of Care Inc., 400 College Road East, Princeton, NJ 08540) the CG4+ cartridge was used for blood gases first, followed by the 6+ for biochemical/electrolyte analysis, with lag time between cartridges recorded. Analytes determined by the CG4+ cartridge included pH, PCO_2, PO_2, sO_2%, base excess (BE), lactate, bicarbonate (HCO_3), and total CO_2 (TCO_2). The 6+ cartridge measured sodium (Na), potassium (K), chloride (Cl), glucose, hematocrit (Hct), and hemoglobin concentration (Hb). Most analytes are measured by the iSTAT system directly, while TCO_2, sO_2%, Hb, and BE were calculated (Paula at al 2008).

After venipuncture, a physical examination and Body Condition Score (BCS) by pectoral muscle profiling on a scale of 1-5 were recorded. Cloacal temperatures were measured in Fahrenheit (4610® thermometer, Measurement Specialties Inc, Dayton, OH), and converted to Celsius to facilitate temperature corrections. All birds were apparently healthy and no clinical findings warranted exclusion of any individual sampled from the study.

Capillary tubes were prepared within 24 hours from the heparinized sample. Blood was spun in lithium heparin microhematocrit tubes at 15,000 g for 3 minutes (StatSpin® centrifuge, Iris® sample processing, Westwood, MA, USA) for packed cell volume (PCV) determination. Indirect white blood cell (WBC) count estimates were made using eosinophil stain (ENG Scientific Inc, Clifton, NJ) to approximate the number of granulocytes. A solution of 0.01 mL of blood and 0.31 mL of eosinophil stain was inverted until mixed. One drop of the resulting solution was placed into a Neubauer Improved hemocytometer (InCyto® C-chip, Republic of Korea) and cells allowed to settle for 5 minutes. Total granulocyte count was obtained by counting the total number of cells within the square grids and multiplying by a factor of 80. Blood smears were prepared from samples without anticoagulant and actively air dried and stored for staining. Blood smears were fixed and stained with modified Wright's stain and the blood monolayer was evaluated for a 100-cell WBC differential count. Within 30 days of sampling, stored plasma samples were thawed and plasma osmolality was measured in duplicate via a commercial freezing-point depression osmometer (Micro-Osmette, Precision Systems, Natick, MA). The osmometer was calibrated utilizing a 2-point calibration technique in accordance with manufacturer instructions. The two osmolality measurements from each patient were used to calculate a single mean value which was then compared with calculated values.

Statistical methods

Data was tested for normality using Analyse-it® Method Evaluation 2.30 add-in software for Microsoft Excel® (Microsoft Office 2010, Analyse-it® Software Ltd, http://www.analyse-it.com/, 2009). Normality for each analyte was assessed by histogram and Shapiro-Wilk statistic ($p>0.05$). Outliers for each analyte were identified and removed via Horn's algorithm and Tukey's interquartile ranges (IQR) as any value that exceeded 1.5 times the (IQR) from the 1st and 3rd quartiles ($< Q_1$-1.5*IQR or $> Q_3$+1.5*IQR), as recommended by the American Society of Veterinary Clinical Pathology when establishing reference intervals (Freidrichs er al 2012). For analytes that exhibited Gaussian distribution, parametric methods were used to create 95% reference intervals. If the results for the analyte had a non-Gaussian distribution, non-parametric methods were used to generate 95% reference intervals. The effect of age, gender, capture type and time of day (Day or night) on the analytes was assessed using Student's t-test for parametric data, and Kruskal-Wallis tests for non-parametric data. Bland-Altman plots were used to assess agreement between: (1) osmolality measured by freezing-point depression (Osm_{MEAS}) and calculated osmolality (Osm_{CALC}); and (2) PCV as measured by centrifugation and Hct as measured by the iSTAT. A 1-way ANOVA test was used to assess the relationship of BCS with the analytes. Regression analysis was used to assess the impact of elapsed capture time in Mottled Ducks. Additional calculation were that osmolality and anion gap were calculated$_{(CALC)}$ with standard formulas while pH, pCO2 and pO2 were temperature corrected $_{(TC)}$ based on cloacal temperatures collected in the field(Paula et al 2008; Steinmetz et al 2007).

RESULTS:

Reference Intervals for use with the iSTAT -1® system were obtained for Black Belly Whistling Ducks, Yellow Crowned Night Herons, Mottled Ducks, Brown Pelicans. The following values were determined: pH (venous), pCO_2 (carbon dioxide partial pressure, venous), pO_2 (oxygen partial pressure, venous), lactate, HCO_3 (bicarbonate), TCO_2 (total CO_2), BE(base excess), SvO_2 (dissolved oxygen, venous), Glu (glucose), BUN (blood urea nitrogen), Hct (calculated hematocrit), Hb (hemoglobin), lactate, AG (anion gap), Na (sodium), K (potassium), and Cl (chloride). Most analytes were parametrically distributed. Sample mean and estimates of the population 95% Reference Interval for each analyte are reported (Table 2). HCT (hematocrit, a measure of the % of red blood cells) as determined by the iSTAT-1® does not reliably predict that determined by the gold standard, the centrifuge. Osmolality measured by osmometer agreed well with values calculated from the equation [2(Na + K) + (GLU/18)]. Body Condition Score, Gender and Age had minimal clinically relevant effect on most analytes (Data not shown).

Table 2: Means of select venous blood analytes of Texas Gulf Coast Birds

Analyte	Units	Mean			
		Mottled Ducks	Yellow Crowned Night Heron	Black Bellied Whistling Duck	Brown Pelican
n	Animal	80-83	81-117	121-129	12
Temp	°C	40.3	39.8	42.0	40.1
pH	37°C	7.42	7.38	7.35	7.37
pCO_2*	mm Hg	31	29.9	32.4	32.9
pO_2	mm Hg	36.9	45.3	39.0	39
sO_2*	%	72	80	71	72
Base Excess	mmol/L	-4.7	-7.6	-7.5	-6.8
Anion Gap	mmol/L	19.3	15.7	20.7	17.5
HCO_3	mmol/L	19.8	17.6	18.0	18.7
TCO_2	mmol/L	20.9	18.6	18.9	19.7
Lactate*	mmol/L	4.97	6.26	7.87	9.40
Na	mmol/L	146	142	142	143
K	mmol/L	3.9	4.2	3.7	3.7
Cl	mmol/L	111	112	109	113
Glucose*	mg/dl	253	231	244	271
BUN¥	mg/dl	<3	<3	<3	<3
Osm_{Meas}	mmol/L	314	299	294	327
Osm_{Calc}	mmol/L	314	305	305	310
Hct	%	36.7	32.6	36.5	40.1
Hgb	g/dl	12.5	11.1	12.4	13.6
PCV*	%	44.5	41.8	45.8	48.4

*non-normal distribution, Shapiro Wilk< 0.05 ¥Most BUN values < 3, given as mode

CONCLUSION:

Reference intervals in tabular format and a point of care analyzer will allow avian health care providers to assess and treat electrolyte and venous blood gas abnormalities in birds affected by oil as well as other maladies necessitating critical care. iSTAT-1® results for HCT should be corrected by increase of approximately 10 percentage points to accurately assess patient status for the coastal bird. Osmolality data can be used to modify currently available fluids for use in avian species during oil spill response. These preliminary findings are hoped to ultimately provide a basis for comparison to birds affected by oil or other common critical maladies so that appropriate fluids can be tailored on a population basis in these incidents. Based on these preliminary findings, appropriate fluid therapy choices to most closely provide a solution of appropriate osmolality and electrolyte range if given intravascularly in birds, of those fluids commonly available to veterinarians would include: Ringer's Solution, Normosol-R (Multisol – R), Normal Saline (0.9%) supplemented with 4 meq of Potassium. The authors' personal preference of these would be Normosol –R as it does not rely on the buffer lactate, the concentration of which may already be increased in birds after undergoing capture. However, we caution the reader that this suggestion is based solely on fluid composition in healthy birds, not electrolyte and acid base changes occurring in the oil affected bird, determination of which should be the gold standard in order to direct fluid therapy toward the normal state.

Hematological, electrolyte, and blood gas analytes determined here provide clinicians with a more reliable means of assessing systemic health, important in petroleum toxicity, as exposure can cause multiple system dysfunctions. Blood gas values are likely to be useful in assessing the respiratory system, while lactate and pH may be valuable to prognose patient outcome. Continuing to establish reference intervals of venous blood analytes from iSTAT-1® system in apparently healthy avian coastal species gives future investigators clinicopathologic parameters to assess health of the Gulf Coast ecosystem. If an oil spill or other environmental disturbance affects the Gulf Coast, a database of normal diagnostic blood parameters will allow biologists to gauge the severity of the disturbance by assessing the health of wildlife. Future recommended investigations of avian blood should include acute phase proteins (fibrinogen, Serum Amyloid A, haptoglobin) as markers of inflammation and recovery as well as the effect of red blood cell lysis, a potential side effect of crude oil ingestion, on these parameters.

REFERENCES:

Abou-Madi N and G.V. Kollias.1992 Avian fluid therapy. In: Kirk R., J. Bonagura, and R. Miller, (Eds.), Current Veterinary Therapy XI. Philadelphia: WB Saunders; 1992:1154-1159

Acierno M.J., A. Cunha, J. Smith, T.N.Tully Jr, D.S.M. Guzman, V. Serra, and M.A. Mitchell. 2008. Agreement between direct and indirect blood pressure measurements obtained from anesthetized Hispaniolan Amazon parrots. Journal of the American Veterinary Medical Association Nov 15 2008, 233(10) pp1587-1590.

Acierno M.J., M.A. Mitchell, D.M. Freeman, P.J. Schuster, D.S.M. Guzman, and T.N.Tully. Determination of plasma osmolality and agreement between measured and calculated values in healthy adult Hispaniolan Amazon parrots *(Amazona ventralis).* American Journal of Veterinary Research 70(9) September 2009: 1151-1154

Deepwater Horizon Bird Impact Data from the DOI-ERDC NRDA Database 12 May 2011. USFWS website accessed June 17 2011. http://www.fws.gov/home/dhoilspill/pdfs/Bird%20Data%20Species%20Spreadsheet%2005122011.pdf

Freidrichs, K.R., K.E. Harr, K. P. Freeman, B. Szladovits, R. M. Walton, K. F. Barnhart, and J. Blanco-Chavez. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Veterinary Clinical Pathololology 2012 Dec;41(4):441-53

Fry, D.M., and L. J. Lowenstein. Pathology of Common Murres and Cassin's Auklets exposed to oil. Archives of Environmental Contamination and Toxicology 1985:14/6 (725-737)

Leighton, F.A. Heinz-Body Hemolytic Anemia from the Ingestion of Crude Oil: A Primary Toxic Effect in Marine Birds. 20 May 1983 Science 20:pp871-873

Leighton, F.A., Morphological Lesions in Red Blood Cells from Herring Gulls and Atlantic Puffins Ingesting Prudhoe Bay Crude Oil Veterinary Pathology 22; 393-402 (1985)

Hernandez M., and R. Aguilar. Steroids and fluid therapy for treatment of shock in the critical avian patient. Seminars in Avian and Exotic Pet Medicine. 1994; 3(4): 190-199.

Paula, V., D. Fantoni, D. Otsuki, J. Auler. Blood-gas and electrolyte values for Amazon parrots *(Amazona aestiva)*, Pesquisa Veterinaria Brasileira [Brazilian Journal of Veterinary Research]. 2008;28(2):108-112.

Stockham, S.L., M.A. Scott Fundamentals of Veterinary Clinical Pathology.2nd edition (2008) Chapter 1. pp 13-15.

Stosskopf M.S., D. M. Mulcahy, D. Esler. Evaluation of a Portable Automated Serum Chemistry Analyzer for Field Assessment of Harlequin Ducks, Histrionicus histrionicus. SAGE-Hindawi Access to Research Veterinary Medicine International Volume 2010, Article ID 418596, 5 doi:10.4061/2010/418596

Steinhort, L. Avian fluid therapy. Journal of Avian Medicine and Surgery. 1999; 13(2): 83-91.

Steinmetz, H., R. Vogt, S. Kastner, B. Riond, J.M. Hatt. Evaluation of the i-STAT portable clinical analyzer in chickens *(Gallus gallus).* Journal of Veterinary Diagnostic Investigation 2007;19:382-388.

Rocke, T.E. USGS Field Manual of Wildlife Diseases - General Field Procedures and Diseases of Birds. Chapter 42 Oil: http://www.nwhc.usgs.gov/publications/field_manual/chapter_42.pdf Accessed June 16, 2011

Tseng, F. Considerations in care for Birds Affected by Oil Spills. Seminars in Avian and Exotic Pet Medicine, 8(1)January 1999: pp 21-31

Zaias, J., W.P. Fox, C. Cray, N.H. Altman. Hematologic, plasma protein, and biochemical profiles of brown pelicans (Pelecanus occidentalis). American Journal of Veterinary Research. 2000 Jul;61(7):771-4