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Hematologic, Biochemical, and Cytologic Findings from Apparently Healthy Atlantic Bottlenose Dolphins (*Tursiops truncatus*) Inhabiting the Indian River Lagoon, Florida, USA

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ABSTRACT: The objective of this study was to establish reference baseline data for hematologic, biochemical, and cytologic findings in apparently healthy Atlantic bottlenose dolphins (*Tursiops truncatus)* inhabiting the Indian River Lagoon, Florida, USA. Sixty-two dolphins were captured, examined, and released during June 2003 and June 2004. Mean, standard deviation, and range were calculated for each parameter, and values for which published data were available, were close to or within the ranges previously reported for free-ranging bottlenose dolphins. No pathologic abnormalities were found in fecal and blowhole cytologic specimens. However, 24% (7/29) of the dolphins examined in 2003 had evidence of gastritis, which was graded as severe in 14% (4/29) of the cases. In 2004, only 4% (1/24) of dolphins sampled had evidence of mild or moderate gastritis; no severe inflammation was present. Dolphins with evidence of gastritis were 8 yr of age or older and predominantly male. Several statistically significant differences were found between males and females, between pregnant and nonpregnant animals, and between juveniles (<6 yr) and adults (≥6 yr). However, the values remained within the established ranges for this species, and the differences were not likely to be of clinical significance.

Key words: Bottlenose dolphin, cytology, haematology, Indian River Lagoon, serum analyte, *Tursiops truncatus*.

The Health and Risk Assessment (HERA) project was initiated in 2003 by the Harbor Branch Oceanographic Institution and the National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, to study the Atlantic bottlenose dolphin (*Tursiops truncatus*) populations inhabiting the Indian River Lagoon (IRL), Florida, USA, and the coastal waters of Charleston, South Carolina, USA. The IRL is the most biodiverse coastal estuary in North America (Gilmore, 1985). It was designated an Estuary of National Significance in 1990 (IRL National Estuary Program, 1996). No comprehensive health assessments of bottlenose dolphins have been conducted within the IRL. A study of IRL dolphins from 1979 to 1981 involved marking and monitoring of marked animals, mostly within the northern section of the IRL (Odell and Asper, 1990). Blood samples were obtained from 109 IRL dolphins over a 3-yr period and tested for a limited panel of hematologic and biochemical parameters (Asper et al., 1990).

Emerging diseases of marine mammals are issues of concern in the IRL (Bossart et al., 2003). Infectious diseases, such as lobomycosis, are present in epidemic proportions (Reif et al., 2005). Serum antibodies against zoonotic disease agents, such as *Toxoplasma* sp. (Dubey et al., 2005) and *Brucella* sp., also have been detected (HERA project, unpubl. data). Between 1993 and 2000, dolphin strandings in the IRL represented approximately 40% of reported bottlenose dolphin strandings along the east coast of Florida (M. Stolen, pers. comm.). Photoidentification data and pathologic findings in stranded dolphins have identified a variety of diseases in IRL dolphins that may have anthropogenic components (Bossart et al., 2003). Several pathologic findings suggest that immunologic dysfunction plays a role in pathogenesis of some diseases. Unfortunately, no definitive explanation currently exists for these patterns, because no

long-term comprehensive studies have assessed the health status of IRL dolphins. Dolphin health and population status in the IRL reflect the effects of natural and anthropogenic stressors and may serve as sentinels for ecosystem health (Bossart, 2005a). Defining the health status of bottlenose dolphins is important for future management of this species and provides an insight regarding the ecosystem as a whole (Bossart, 2005b). The current study was designed to establish baseline values for hematologic, biochemical, and cytologic parameters for long-term use in monitoring the population of IRL dolphins.

Sixty-two Atlantic bottlenose dolphins were captured, examined, sampled, marked, and safely released from the IRL in June 2003 and June 2004. The study area extended from the north near Merritt Island, Florida, USA (28°29′52.73N 80°38′00.91W) to the south in the St. Lucie Inlet (27°9′59.68″N 80°9′30.13″W). Dolphins were collected by encircling them with a net in less than 2 m of water to facilitate safe handling. Dolphins were restrained in the water for collection of blood samples and then moved to a processing boat for the remainder of the sample-collection processes. Each animal received a complete physical and ultrasound examination to determine pregnancy status. Dolphins exhibiting external signs of ill health (e.g., extensive skin lesions, orogenital papillomatous lesions, and emaciated body condition) were excluded. Age was determined by extracting a tooth and examining dental enamel according to the procedures described by Hohn et al. (1989). Age was not determined for 13 of the dolphins released for a variety of reasons, including dangerous weather conditions, pregnancy status, and excessive distress. Six animals captured during 2003 were recaptured in 2004. Only data from the initial capture were included for each dolphin. The Institutional Animal Care and Use Committee of the Harbor Branch Oceanographic Institution approved all animal capture and sampling protocols.

Blood samples were drawn from the periarterial venous rete in the flukes within the first 10 min of capture with a 19-gauge, 1.9-cm, butterfly catheter (Becton Dickinson, Franklin Lakes, New Jersey, USA) (Bossart et al., 2001). Serum was collected in 10-ml separator vacutainer tubes (Becton Dickinson), placed in a cooler for between 20 and 40 min, and centrifuged for 15 min at 1,200 rpm. Samples for hematology were collected in a vacutainer tube with ethylenediaminetetraacetic acid as an anticoagulant (Becton Dickinson). Samples were stored in an insulated cooler and shipped overnight to the Cornell University Veterinary Diagnostic Laboratory, Ithaca, New York, USA.

A complete blood count was done in 59 dolphins. Relative leukocyte determinations were performed by microscopic examination of modified Wright-stained blood smears (Bayer Healthcare, Tarrytown, New York, USA). A microhematocrit tube was centrifuged for 5 min at 11,700 rpm, and the spun hematocrit was interpreted by visual inspection against a standard calibration. Automated hematocrit, hemoglobin, red blood cell count, mean corpuscular platelet volume, mean corpuscular hemoglobin (MCH), MCH concentration, red cell distribution width, white blood cell count, platelets, and mean platelet volume were determined by an automated analyzer (Bayer ADVIA 120, Bayer Diagnostics, Tarrytown, New York, USA). The concentrations of serum chemistry analytes were determined in 62 dolphins with an automated analyzer (Hitachi 917, Roche, Indianapolis, Indiana, USA) and included analyses for sodium, potassium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphate, magnesium, total protein, albumin, globulin, albumin:globulin ratio, glucose, total direct and indirect bilirubin, cholesterol, triglycerides, iron, total iron-binding ca-

pacity (TIBC), percentage saturation (PSAT), and fibrinogen. Enzyme activity was determined for alanine aminotransferase, alkaline phosphatase, amylase, aspartate aminotransferase, creatinine phosphokinase (CPK), gamma-glutamyltransferase, lactate dehydrogenase, and lipase. Serum protein electrophoresis (SPEP) was conducted with an automated analyzer (Rapid Electrophoresis, Helena Laboratories, Beaumont, Texas, USA). Fibrinogen concentration was determined by the method of Schalm using heat precipitation. Hemolyzed or lipemic samples were not included.

Swabs for cytologic evaluation were collected from the blowhole, feces, and gastric fluid from 53 dolphins (Sweeney and Reddy, 2001). A sterile culture swab (MML Diagnostics Packaging, Troutdale, Oregon, USA) was inserted into the blowhole during a breath, gently moved around the wall, and removed during the subsequent breath. The swab was then used to prepare a thin smear on glass slides (VWR Scientific Products, West Chester, Pennsylvania, USA).

Gastric samples were collected by inserting a lubricated, flexible plastic tube (Kalayjian Industries Inc., Long Beach, California, USA) into the forestomach. Fluid was collected by aspiration into 15-ml Falcon conical centrifuge tubes (BD Biosciences, San Jose, California, USA) (Bossart et al., 2001). Direct examination of the gastric fluid was performed by examining thin smears on glass slides (Sweeney et al., 2003). Fecal samples were collected by inserting a sterile swab into the anus and gently swabbing the area. The sample was then thinly smeared on a glass slide.

Slides for cytologic evaluation were air-dried and stained with Wright-Giemsa (Jorgensen Laboratories Inc., Loveland, Colorado, USA). Samples were examined for morphologic preservation, overall cellularity, presence and type of epithelial cells, inflammatory cell types and degree of inflammation, as well as presence of bacteria, fungi, parasites, and noncellular debris (Sweeney et al., 2003). Ten 400× fields were examined and assigned an average for each parameter.

Descriptive statistics were calculated for the hematologic, serum chemistry, and SPEP data using Microsoft Excel 2003 (Microsoft Corp., Redmond, Washington, USA). Comparisons between males and females, pregnant and nonpregnant females, and juveniles (<6 yr) and adults (≥6 yr) were performed using one-way analysis of variance (ANOVA), with $P<0.05$ as the level for statistical significance.

Sixty-two clinically healthy dolphins were included in the analysis (38 males, 20 nonpregnant, and four pregnant females). Hematologic data were available for 59 animals. The ages of 49 animals were determined from examination of dental enamel (Hohn, 1989) and ranged from 3.5 to 26 yr, with a mean age of 12.4 yr for males and 9.1 yr for females. Overall, 42 adults (≥6 yr; 11 females and 31 males) and seven juveniles (<6 yr; five females and two males) were sampled.

Sample size, means, standard deviations, and ranges for hematologic parameters are shown in Table 1. Females had significantly higher mean spun hematocrit ($P<0.001$) values compared with males. No statistically significant differences ($P>0.05$) were found in any hematologic parameters between pregnant and nonpregnant females. Juvenile animals had a higher mean MCH ($P=0.04$) and a significantly lower relative percentage of segmented neutrophils ($P=0.05$) compared with adults.

Serum biochemical and SPEP parameters are shown in Table 2. Females had higher concentrations than males for sodium ($P=0.02$), BUN ($P=0.03$), BUN:creatinine ratios ($P=0.03$), CPK ($P=0.02$), cholesterol ($P=0.02$), triglycerides ($P=0.005$), PSAT ($P=0.04$), and iron ($P=0.03$). Males had a higher mean lipase activity ($P=0.04$) than females. Nonpregnant females had significantly higher

TABLE 1. Hematology values for Atlantic bottlenose dolphin population in the Indian River Lagoon, 2003–04.

	Unit of measure[a]	Mean	SD	Range		n
				Min.	Max.	
White blood cells	10^3/μl	10.31	2.58	5.8	19.5	59
Red blood cells	10^6/μl	3.61	0.28	2.8	4.8	59
Hemoglobin	g/dl	14.47	1.11	11.3	18.2	59
Manual hematocrit	%	40.15	2.41	35	46	26
Automated hematocrit	%	40.37	3.03	32	50	59
Mean corpuscular volume	fl	112.2	6.46	96	126	59
Mean corpuscular hemoglobin	pg	40.41	2.55	33	45	59
Mean corpuscular hemoglobin concentration	g/dl	35.93	1.01	32	38	59
Red cell distribution width	%	12.45	1.01	11.1	16.1	59
Basophils	10^3/μl	0.04	0.08	0	0.3	59
Segmented neutrophils, relative	% of WBCs	44.55	9.89	25.35	68.37	59
Segmented neutrophils, absolute	10^3/μl	4.6	1.65	1.8	12.7	59
Bands-relative	% of WBCs	0.04	0.31	0	2.41	59
Bands-absolute	10^3/μl	0	0.03	0	0.2	59
Lymphocytes, relative	% of WBCs	19.29	7.8	2.04	47.33	59
Lymphocytes, absolute	10^3/μl	1.96	0.93	0.2	6.2	59
Monocytes, relative	% of WBCs	3.29	2.24	0	10.69	59
Monocytes, absolute	10^3/μl	0.35	0.31	0	1.6	59
Eosinophils, relative	% of WBCs	32.4	9.28	13.68	52.75	59
Eosinophils, absolute	10^3/μl	3.35	1.27	1.3	7.1	59
Nucleated red blood cells		0.5	0.71	0	1	59
Platelets	10^3/μl	167.12	41.68	73	281	59
Mean platelet volume	fl	12.12	2.21	8.6	20.6	59
Total protein, refractometer	g/dl	7.62	0.51	6.6	9.7	59
Fibrinogen	mg/dl	138.14	89.22	50	400	59

[a] WBCs = white blood cells.

mean TIBC ($P=0.003$) and albumin ($P=0.04$) than pregnant females. Juveniles had higher mean values than adults for BUN ($P=0.04$), BUN:creatinine ratio ($P=0.03$), CPK ($P=0.04$), triglycerides ($P=0.05$), iron ($P<0.01$), PSAT ($P=0.01$), and lipase activity ($P=0.02$).

No evidence of an inflammatory response or other cellular abnormalities was found in the cytologic preparations made from fecal and blowhole swabs. However, 24% (7/29) of the dolphins examined in 2003 had evidence of neutrophilic gastric inflammation (3/29 had mild gastric inflammation, and 4/29 had severe gastric inflammation). In 2004, only 4% (1/24) of the population had mild or moderate neutrophilic gastric inflammation; no severe inflammation was present. Findings were not the result of differences in age or sex between dolphins sampled in 2003 and 2004. All animals exhibiting gastric inflammation were 8 yr of age or older (average age, 14 yr). All but one animal with gastric inflammation was male.

Data for several hematologic and serum biochemical parameters have been reported previously for IRL dolphins (Asper et al., 1990); for free-ranging dolphins captured in Sarasota Bay, Florida, USA (Bossart et al., 2001); and for dolphins living in coastal enclosures in the Pacific Ocean (Ridgway et al., 1970). However, none of these previous studies included the extensive set of parameters measured and reported in the present study. The current study also included cytologic findings, which to our knowledge have not been described previously for IRL dolphins. In addition to establishing mean

TABLE 2. Serum biochemical and serum protein electrophoretic values for Atlantic bottlenose dolphin population in the Indian River Lagoon, 2003–04.

	Unit of measure	Mean	SD	Range		*n*
				Min.	Max.	
Serum biochemistry						
Glucose	mg/dl	93.87	12.14	66	122	62
Sodium	mEq/l	155	2.44	151	164	62
Potassium	mEq/l	3.97	0.32	3.4	4.8	62
Sodium/potassium	mEq/l	39.73	3.19	33	44	26
Chloride	mEq/l	113.47	2.91	106	119	62
Bicarbonate	mEq/l	20.69	4.01	9	28	62
Anion gap	mEq/l	24.87	5.33	17	40	62
Blood urea nitrogen (BUN)	mg/dl	66.29	9.48	46	87	62
Creatinine	mg/dl	1.09	0.27	0.6	1.8	62
BUN:creatinine ratio	mg/dl	65	19.21	25.56	110	62
Total protein	g/dl	7.45	0.58	6.4	10	62
Albumin	g/dl	4.49	0.33	3.2	5.2	62
Globulin	g/dl	2.96	0.68	2	6.8	62
Albumin:globulin ratio	g/dl	1.59	0.34	0.47	2.48	62
Total bilirubin	mg/dl	0.09	0.03	0	0.2	62
Direct bilirubin	mg/dl	0.02	0.04	0	0.1	62
Indirect bilirubin	mg/dl	0.07	0.05	0	0.1	62
Calcium	mg/dl	9.33	0.48	8.3	10.5	62
Phosphorus	mg/dl	5.2	0.7	3.6	7.1	62
Magnesium	mg/dl	1.44	0.14	1.2	1.9	62
Uric acid	mg/dl	1	0.52	0.2	2.3	26
Alkaline phosphatase	U/l	272.39	149.58	43	899	62
Alanine aminotransferase	U/l	44.42	23.39	19	122	26
Aspartate aminotransferase (AST)	U/l	255.89	91.96	142	733	62
Sorbital dehydrogenase (SDH)	U/l	10.53	10.83	1	51	59
Lactate dehydrogenase (LDH)	U/l	493.38	83.94	378	706	26
Creatinine protein kinase (CPK)	U/l	152	41	82	291	62
Amylase	U/l	1.12	0.43	1	3	26
Lipase	U/l	10.35	4.6	4	27	26
Gamma glutamyltransferase (GGT)	U/l	27.45	3.9	17	39	62
Cholesterol	mg/dl	140.23	25.48	88	183	26
Triglyceride	mg/dl	83.03	32.42	41	182	62
Iron	μg/dl	98.85	35.07	32	206	62
Total iron binding capacity (TIBC)	μg/dl	249.18	62.41	174	579	62
Percent transferrin saturation (PSAT)	%	40.24	12.3	15	73	62
Serum protein electrophoresis						
Total protein	g/dl	7.45	0.58	6.4	10	62
Albumin	g/dl	3.75	0.31	3.05	4.64	62
Total globulin	g/dl	3.69	0.66	2.67	6.92	62
Total α-globulins	g/dl	1.19	0.24	0.68	1.81	62
α_1-Globulin	g/dl	0.35	0.2	0.11	0.84	62
α_2-Globulin	g/dl	0.85	0.18	0.37	1.17	62
Total β-globulins	g/dl	0.45	0.1	0.31	0.74	62
β_1-Globulin	g/dl	0.27	0.12	0.12	0.57	45
β_2-Globulin	g/dl	0.21	0.05	0.15	0.39	36
Total γ-globulins	g/dl	2.05	0.61	0.83	5.18	62
Albumin:globulin ratio	g/dl	1.05	0.22	0.44	1.74	62

baseline values for each parameter, the effects of sex, age, and pregnancy status were examined to determine their potential relationships to each parameter. Sex- and age-related differences have been described previously in captive bottlenose dolphins for some hematologic and biochemical parameters (Asper et al., 1990). We compared IRL mean hematologic and serum analyte values to previously published ranges for free-ranging dolphins as well as for IRL dolphins (Asper et al., 1990; Bossart et al., 2001). All values were very close to or within the previously published ranges for these parameters.

Caution must be used when interpreting the results of differences between juveniles and adults as well as between pregnant and nonpregnant females. Our sample sizes for pregnant females and juvenile dolphins were small and may not have been representative of the entire population because of constraints imposed by the permit process. Second, in conducting statistical analyses for a large set of parameters by age, sex, and pregnancy status, we performed many comparisons. The results of some of these comparisons likely are the result of chance. Most important, all the significant findings in this study involved values considered to be in the clinically normal range. Therefore, these findings are of interest, but they are not likely to be of clinical significance.

As mentioned, several of the statistically significant findings are of interest. The spun hematocrit was higher among females than among males, but the hematocrit was not different when determined by autoanalyzer. The spun microhematocrit method has been recommended for clinical samples because of the potential for electronic cell counter error (Bossart et al., 2001). Elevated hematocrit values often are indicators of dehydration; in addition, the hematocrit can increase by as much as 23% in seals following stress (Medway and Geraci, 1986). Female dolphins may be subjected to greater stress levels than males during capture, especially when accompanied by a calf. Two cow/calf pairs were processed in each year. However, other changes characteristic of stress were not observed in the leukogram, hematocrit values remained within the range of values considered to be clinically healthy, and sex-related differences in hematocrit values have not been described in other dolphin populations.

Although adults had a higher relative percentage of neutrophils compared with juveniles, the absolute white blood cell counts were not significantly different ($P>0.05$) between the two groups. Differences in neutrophil percentages were similar to findings for captive dolphins (Asper et al., 1990).

Differences in concentrations of several biochemical parameters, including iron, were found between males and females. Sex-related differences were reported in a previous study of captive dolphins in which females also had higher iron and TIBC values compared with those in males (Asper et al., 1990). With the exception of serum iron, sex-related differences in biochemical parameters have not been demonstrated in other wild dolphin populations. Juvenile dolphins had significantly higher mean values than adults for BUN, BUN : creatinine ratio, triglycerides, iron concentrations, PSAT, CPK, and lipase enzymatic activities. Higher CPK activities have been described previously in captive juvenile bottlenose dolphins compared to those in adults (Asper et al., 1990). Creatinine phosphokinase is present in many tissues and is elevated primarily with skeletal muscle damage. Younger dolphins may have had increased skeletal muscle damage as a result of handling compared to adults.

Because only clinically healthy animals were selected for this study, the gastric inflammation that was found may indicate subclinical disease. Marine mammals often mask early signs of poor health, and disease processes may produce cytologic

abnormalities that precede the onset of clinical signs (Cowell et al., 1999; Sweeney and Reddy, 2001). The high prevalence of severe gastric inflammation in our 2003 samples may be an indicator that pathologic stressors were affecting these individuals. The reasons responsible for the preponderance of gastric inflammation among older males are unclear.

The extensive set of hematologic, biochemical, protein electrophoresis, and cytologic parameters described in this study can be used as a screening tool for health assessments (Reif et al., 2004). In the current study, values for these parameters fell, with few exceptions, within the ranges described in previous studies of captive and free-ranging bottlenose dolphins. Our data suggest that the "apparently healthy" IRL dolphins also have clinicopathologic parameters of a healthy animal. We are currently examining a comprehensive battery of diagnostic tests and biomarkers performed during the HERA study. It is imperative to include physical examination, history, and diagnostic testing to obtain an accurate assessment of marine mammal health (Bossart et al., 2001). Thus, when combined with the results of other current and previously conducted research, the data from this study should serve as a reference point for further integrated health studies of Atlantic bottlenose dolphins.

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