Aquatic Mammals 2009, *35*(2), 253-258, DOI 10.1578/AM.35.2.2009.253

Blood Chemistry of Antillean Manatees (*Trichechus manatus manatus*): Age Variations

Fernanda M. O. Silva,[1] Jociery E. Vergara-Parente,[1] Jeane K. N. Gomes,[2] Miriam N. Teixeira,[1] Fernanda L. N. Attademo,[2] and Jean Carlos R. Silva[1,3]

[1]*Programa de Pós-Graduação em Ciência Veterinária – PPGCV, Departamento de Medicina Veterinária – DMV, Universidade Federal Rural de Pernambuco – UFRPE, Recife, Pernambuco, Brazil; E-mail: fernanda.pxboi@gmail.com*
[2]*Fundação para Preservação e Estudos dos Mamíferos Aquáticos – FMA, Recife, Pernambuco, Brazil*
[3]*Instituto Brasileiro para Medicina da Conservação – Tríade, São Paulo, São Paulo, Brazil*

Abstract

The Antillean manatee (*Trichechus manatus manatus*) is the most endangered aquatic mammal in Brazil. Sampling blood data from such critically endangered marine mammal species is extremely challenging. Although several hematological studies have been developed for captive manatees, captivity studies addressing the environmental and physiological effects on blood values are scarce. The present work describes blood biochemistry values for captive Antillean manatee adults and calves and verifies the occurrence of possible physiological adjustments due to age, sex, and dietary influences. Blood from 13 clinically healthy manatees (eight calves and five adults) were analyzed for 13 blood serum chemistry parameters using a semi-automatic analyzer. Descriptive analysis was performed for all parameters, and differences between sex and age were determined. Calves had higher means of urea (6.29 ± 5.58 mg/dL), total proteins (5.07 ± 0.94 g/dL), globulin (3.06 ± 1.32 g/dL), and alanine aminotransferase (6.19 ± 2.18 U/mL), levels, and lower means of creatinine (1.42 ± 0.64 mg/dL), aspartate aminotransferase (7.24 ± 3.21 U/mL), phosphate (3.03 ± 1.63 mg/dL), and uric acid (0.71 ± 0.17 g/dL) than adults. Further studies are necessary, especially when considering handling and alimentary management in captivity, to provide important data for better monitoring and clinical management of manatees.

Key Words: Antillean manatee, *Trichechus manatus manatus*, serum chemistry, captive, blood

Introduction

The clinical laboratory has been extended to wildlife medicine as a tool for the prevention, diagnosis, and control of diseases in marine mammals (Almosny & Monteiro, 2006). Some species can be considered as environmental indicators of health problems, which can be extremely valuable for wild animals kept in captivity for long periods. Through the observation of sentinel animals, it is possible to assess and monitor several parameters of an ecosystem's health, thus evaluating which environmental factors influence life in the wild (Reddy et al., 2001).

The development of new analysis techniques and modern equipment has contributed to the improvement of clinical pathology in past decades. Hematology and blood chemistry exams, allied to a well-done anamnesis, complement the clinical evaluation and help diagnose certain diseases (Fowler, 1986; Farver, 1997). In aquatic mammals, laboratory clinical medicine has been developed and used increasingly. Data obtained during several years of blood analysis allowed some researchers to establish reference intervals for hematological parameters and blood serum analytes (Bossart et al., 2001; Harvey et al., 2009).

Marine mammal researchers have demonstrated the importance and relevance of using a blood chemistry analysis as an index of animal health and nutritional status. It is extremely important to consider and comprehend the relationship between biological factors, such as age, sex, and dietary intake with blood chemistry profiles in marine mammals. Acquiring blood chemistry information from healthy marine mammals can provide valuable information relative to the contribution of internal influences on specific physiological responses. However, the sampling and analysis of blood data from captive animals must also take into consideration the captivity management conditions since external influences may also affect blood parameters significantly.

Sampling blood data from populations of critically endangered marine mammal species is extremely challenging. In manatees (*Trichechus manatus manatus*), the most endangered Brazilian aquatic mammal (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA], 2001), the analyses of several blood parameters have been developed over the past decade (Medway et al., 1982a, 1982b, 1982c). In the last few years, some of these studies have considered the environment and handling conditions at the time of the sample. The methods of routine management procedures can affect some blood chemistry analytes in both free-ranging and captive West Indian manatees (Irvine et al., 1980). Harvey et al. (2007) collected important data regarding variations in blood values with respect to location, age class, and sex on plasma of healthy Florida manatees. Although some reports describe the variations due to changes in management procedures (Manire et al., 2003), there are very few studies carried out on captive manatees addressing the importance of environment and physiological factors. Therefore, the objective of this work was to describe blood biochemistry values for captive Antillean manatee adults and calves, describing possible physiological adjustments that may exist due to age, sex, and dietary intake.

Material and Methods

Only clinically healthy manatees were included in this study. Their health was confirmed by external visual evaluation, behavior assessment (Reidarson et al., 2000), and that they were neither visibly pregnant nor under any medication or treatment during the analysis period. All individuals were subjected to similar handling and human contact.

Captive Antillean manatees were assigned to groups according to age and sex. The division according to the body length measurements was not possible due to the restrictions that captivity may exert on the animals' development since the oceanaria where they were kept was near its maximum capacity. Therefore, four groups were identified: (1) male calves, (2) female calves, (3) male adults, and (4) female adults. Calves were defined as animals from birth to 3 y of age and adults as the animals older than 7 y. Based on this classification, it was possible to carry out research on eight calves (four males and four females) and five adults (two males and three females), totaling 13 manatees.

All the calves and adults were rescued from stranding events and kept in captivity at the Centro Mamíferos Aquáticos – CMA/ICMBio. The diet of orphaned calves was primarily composed of soy milk hand-rearing formulas (Aptamil® Soja 2 – Support, São Paulo, São Paulo, Brazil; and Soymilke – Olvebra Industrial S/A, Eldorado do Sul, Rio Grande do Sul, Brazil), while the adults' diet was composed of seagrass (*Halodule wrightii*) and complemented with carrots (*Daurus carota*).

Samples were obtained thorough 2005 and 2006 during the routine handling procedures performed by the CMA/ICMBio veterinarians. After physical restraint with a stretcher, blood was drawn from the brachial vascular bundle and placed in serum separator tubes (Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood samples collected into serum separator tubes were allowed to clot for approximately 15 to 30 min. The serum was separated by centrifugation and stored at -20° C until analysis, which took place within 7 d of collection. Samples that appeared icteric, haemolytic, or lipemic were not included in the analysis (Farver, 1997).

All blood chemistry analyses were conducted at CMA/ICMBio Laboratory and were performed using a semi-automatic analyzer (Labquest®; Labtest Diagnóstica S.A., Lagoa Santa, Minas Gerais, Brazil). The enzymatic systems were performed at 37° C. Reagent kits were used to evaluate the parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, glucose, triglycerides, cholesterol, total protein, albumin, phosphate, chloride, calcium, and uric acid. Globulin concentration (subtraction of albumin from total protein) was manually calculated.

All data were entered into an electronic worksheet program (*Microsoft Office Excel 2003*, Microsoft Corporation, Redmond, WA, USA). Statistical analysis was performed using statistical software (*STATISTICA 7 for Windows*, StatSoft, Inc., Tulsa, OK, USA). Descriptive statistics were performed for chemistry parameters (maximum, minimum, mean, and SD). Minimum-maximum values were used as the reference intervals. Data distribution (normal or non-normal) was assessed using the Kolmogorov-Smirnov test. Differences between sex and age were determined using an unpaired Student's t-test. Values with a $p < 0.05$ were considered statistically significant.

Results

The nutritional composition of the two artificial formula offered to the orphaned calves ($n = 8$) and of the sea grass offered to the adults ($n = 5$) are described in Tables 1 & 2. There was no difference in the groups related to the two formulas used during the study period ($p < 0.05$).

There were no significant differences between males and females in both age classes (calves and adults). When compared to the calves, the adults presented higher means for the analytes' total proteins (5.07 ± 0.94 g/dL, $p < 0.05$, $t = -2.81$), globulin (3.06 ± 1.32 g/dL, $p < 0.05$, $t = -2.70$), and ALT (6.19 ± 2.18 U/mL, $p < 0.05$, $t = -3.06$); and lower means for the parameter BUN (6.29 ± 5.58 mg/dL, $p < 0.05$, $t = 4.71$), creatinine (1.42 ± 0.64 mg/dL, $p < 0.05$, $t = 3.07$), AST (7.24 ±

Table 1. Nutritional composition of the artificial milk formulas offered to captive Antillean manatee (*Trichechus manatus manatus*) orphaned calves at CMA/ICMBio Rehabilitation Area, Brazil

	Artificial formula (100 g)	
Compound	Aptamil® Soja 2	Soymilke®
Biotin (mcg)	2.0	3.8
Calcium (mg)	93.0	138.5
Calories (Kcal)	71.0	75.0
Carbohydrates (g)	7.6	6.5
Chlorite (mg)	63.0	--
Cholesterol (mg)	0.0	0.0
Copper (mg)	0.04	0.2
Fatty acids	--	--
Folic acid (mcg)	11.0	26.5
Iodine (mcg)	17.0	14.3
Iron (mg)	1.2	1.9
L-Methionine (g)	--	0.1
Lactose (g)	0.0	0.0
Linolenic acid (an omega-6 fatty acid) (g)	4.4	2.0 g
Linoleic acid (omega-3 fatty acid) (g)	0.8	0.3 g
Magnesium (mg)	8.0	37.5
Manganese (mg)	--	0.3
Monounsaturated (g)	--	1.0
Niacin (mg)	0.4	1.7
Pantothenic acid (mg)	0.3	0.4
Phosphorus (mg)	63.0	87.0
Polyunsaturated (g)	--	2.0
Potassium (g)	100.0	--
Proteins (g)	2.2	3.5
Saturated (g)	1.5	0.5
Sodium (mg)	34.0	15.0
Total fats (g)	3.6	3.5
Vitamin A (mcg)	78.0	49.9
Vitamin B1 (mg)	0.04	0.2
Vitamin B2 (mg)	0.1	0.2
Vitamin B6 (mg)	0.04	0.2
Vitamin B12 (mcg)	0.2	0.2
Vitamin C (mg)	8.6	4.0
Vitamin D (mcg)	1.9	1.8
Vitamin E (mg)	0.8	1.1
Vitamin K (mcg)	6.3	10.4
Water (g)	--	--
Zinc (mg)	0.8	1.5

3.21 U/mL, $p < 0.05$, $t = 2.00$), phosphate (3.03 ± 1.63 mg/dL, $p < 0.05$, $t = 2.94$), and uric acid (0.71 ± 0.17 g/dL, $p < 0.05$, $t = 2.99$) (Table 3).

Discussion

The albumin:globulin ratio (A:G) has been widely used in the recognition of generalized inflammatory diseases. Physiological variations are commonly documented in pregnant mammals, during lactation, and in those involved in stressful situations. Hormonal, sexual, and dietary influences also should be considered (Kaneko, 1997). Albumin, an analyte intimately diet-related, can be easily affected by minimal changes in the animals' feeding. However, aquatic mammals appear to have an elevated hepatic production of this protein-fraction, and in cases of nutritional deficiencies, the serum levels of this analyte would be little affected (Bossart et al., 2001). At birth, total protein (TP) levels are reduced due to the low immunoglobulin (Ig) neonate blood. From the

Table 2. The nutritional composition of sea grass (*Halodule wrightii*) in diet fed to captive Antillean manatees

Compounds	Quantity present in dry material (100%)*
Calcium (%)	1.02
Ethereal extract (%)	2.12
Fiber in acid detergent (%)	48.51
Fiber in neutral detergent (%)	66.60
Mineral material (%)	20.67
Phosphorus (%)	0.02
Raw energy (Kcal/kg)	2,436
Raw protein (%)	10.56

*Magalhães & Leça (2001)

colostrum administration, the TP levels increase due to the ingestion of maternal Igs, which are gradually replaced by the Ig produced by the individual immune system (Kaneko, 1997).

The adult manatees evaluated presented lower levels of BUN and creatinine levels. These results should be carefully evaluated since creatinine levels rise with age and/or muscle mass. Since the animals were fed only with sea grass containing high levels of raw protein, higher levels of BUN were expected. Further analyses are necessary. These kidney function indicators are commonly higher in marine mammals when compared with terrestrial mammals, probably reflecting its food habit differences (Finco, 1997). However, these analyte values are lower in manatees than in other aquatic mammal species, mostly due to the manatees' alimentary habits and particular anatomic features. They are strictly herbivores, being able to produce high concentrated urine (Hill & Reynolds, 1989). Another unique anatomical characteristic for the species is the fact that part of the digestion process happens in its lower gut (Reynolds & Rommel, 1996), similar to what is observed in ruminants and equines.

The importance of uric acid levels is questionable. Some researchers use this analyte as a dehydration indicator, according to what has been verified in some species of dolphins and pinnipeds (Bossart et al., 2001). Although the present study is the first to report its value for captive Antillean manatee calves, further studies are necessary to evaluate its importance as a function marker.

Although the transaminases' role as indicators of hepatic disorder has been studied in some marine mammal species, these enzymes are not liver-specific in manatees (Bossart et al., 2001). AST is commonly used to evaluate muscular damage, especially in cases of capture myopathy. However, these enzymes' activity measurement appears to be compromised by blood isoenzymes since animals with acute or chronic muscle injuries presented no increase in these transaminases levels (Harr et al., 2008).

Serum phosphate values were superior in calves when compared to the adults. This fact can be

Table 3. Comparison between serum chemistry values for captive Antillean manatee calves and adults held at CMA/ICMBio Rehabilitation Area, Brazil

	Orphaned calves (n = 8) Males (n = 4) and females (n = 4)				Adults (n = 5) Males (n = 2) and females (n = 3)			
Parameters	n	Mean	SD	Range*	n	Mean	SD	Range*
Albumin (g/dL)	73	1.91[a]	0.87	0.83 - 6.45	25	2.01[a]	0.58	0.93 - 3.30
ALT (U/mL)	73	4.40[a]	2.78	0.00 - 10.00	21	6.19[b]	2.18	5.00 - 10.00
AST (U/mL)	72	7.38[a]	3.65	0.00 - 20.00	20	5.50[b]	3.94	0.00 - 15.00
Calcium (mg/dL)	72	9.37[a]	1.75	1.79 - 11.84	25	9.47[a]	2.20	4.61 - 13.56
Chloride (mEq/L)	63	84.38[a]	28.01	27.00 - 227.00	21	74.14[a]	20.57	26.00 - 99.00
Cholesterol (mg/dL)	69	51.14[a]	29.68	12.00 - 126.00	24	40.54[a]	15.80	23.00 - 93.00
Creatinine (mg/dL)	76	1.88[a]	0.68	0.50 - 3.90	25	1.42[b]	0.64	0.40 - 2.80
Globulin (g/dL)	76	2.38[a]	0.96	0.24 - 5.78	25	3.06[b]	1.32	0.40 - 6.37
Glucose (mg/dL)	77	59.93[a]	20.36	20.00 - 133.00	22	58.32[a]	17.53	14.00 - 98.00
Phosphate (mg/dL)	56	6.58[a]	4.85	2.00 - 25.10	17	3.03[b]	1.63	0.80 - 8.60
Total proteins (g/dL)	85	4.34[a]	1.16	2.20 - 8.20	25	5.07[b]	0.94	3.70 - 7.30
Triglycerides (mg/dL)	70	68.46[a]	33.31	20.00 - 169.00	24	74.88[a]	38.95	33.00 - 171.00
Urea (mg/dL)	72	16.59[a]	10.79	3.00 - 47.00	25	6.29[b]	5.58	1.00 - 20.00
Uric acid (g/dL)	65	0.97[a]	0.41	0.40 - 2.60	24	0.71[b]	0.17	0.50 - 1.10

n = number of analyzed samples; SD = standard deviation
*Minimum and maximum values
[ab]Different letters in the same line indicate values statistically different ($p < 0.05$)

justified by two physiological factors: (1) age and (2) diet. First, while the adults ingest only 0.004% of phosphorus in 100% of dry green material (Table 2), the calves ingest 63.00 to 87.00 mg of this compound in 100 mL of soy powdered-milk (Table 1). Second, the age factor can exert huge influences on the analyte serum levels since in young animals the growth hormone (GH) leads to a higher phosphorus renal absorption, raising the serum phosphate levels (Rosol & Capen, 1997).

Several hematological studies have been performed for manatees, but long-period studies are still necessary. Although, the values for the marine manatee (White et al., 1976; Medway et al., 1982a, 1982b, 1982c) and its subspecies inhabiting Florida (*Trichechus manatus latirostris*) are well-defined (Bossart & Dierauf, 1990; Bossart et al., 2001), data for other sirenian species are considered scarce (Allen et al., 1976; Colares et al., 1992; Converse et al., 1994; Rosas et al., 1999). Recently, Silva et al. (2007) reported serum chemistry values for captive Antillean manatee adults, not verifying differences between genders. Further studies performed by Harvey et al. (2007) evaluated the effects location (free-ranging vs captive), age class (small calves, large calves, subadults, adults), and sex exert on blood parameters, verifying differences in blood exams of captive animals, suggesting that factors such as diet, stress, and temperature might account for some of these results.

Although Harvey et al. (2007) measured serum chemistry analytes in plasma, their results corroborate with the ones of the present study: total globulins increase while phosphates decrease with age, and AST levels were higher in calves than in adults. Our study is the first to report serum chemistry values for *T. m. manatus* in Brazil, representing an important contribution for a better understanding of the importance of marine mammals' clinical laboratory medicine to Rehabilitation Centers such as CMA/ICMBio, especially the ones which held the animals under their care for long periods. Although the samples size may appear small (five adults and eight calves), this number can be considered significant for the Antillean manatee in Brazil since its population is limited to only 500 individuals (Lima, 1997). Further studies are necessary, especially considering the handling and alimentary management in captivity, factors which influence significantly several serum biochemistry analytes and can provide important data for a better monitoring of the clinical marine mammals' management routine.

Acknowledgments

This study would not be possible without all of the CMA/ICMBio crew and the financial support of Fundo Nacional do Meio Ambiente – FNMA, Ministério do Meio Ambiente, Federal Government. We also thank Dr. Daíza Lima and Msc Ana Carolina Valladares for all the logistical support and assistance during the data statistical analysis.

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