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Changes in the blood parameters of the Amazonian manatee (*Trichechus inunguis*) after long-distance transportation

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ABSTRACT. In this study we report the hematological, biochemical and hormonal parameters in a juvenile male Amazonian manatee measured before transport, immediately after transport, and during adaptation to a new facility. The animal was transported from Manaus, Amazonas State, Brazil, to São Paulo, São Paulo State, Brazil, (2,733 km) within 6 hours. Among all blood parameters analyzed, we observed obvious neutrophilia, lymphopenia, and increases in the neutrophil/lymphocyte ratio and serum glucose and aspartate aminotransferase (AST) levels, but these parameters subsequently returned to normal. These results suggest that transport and changes in the environment are temporary stressful events for Amazonian manatees. We, therefore, recommend monitoring the hematological and biochemical parameters before and after translocation to minimize the effects of handling stressors in this species.

Keywords: sirenian, hematology, blood chemistry, cortisol, stress.

Alterações nos padrões sanguíneos do peixe-boi da Amazônia (*Trichechus inunguis*) após transporte de longa distância

RESUMO. Neste estudo, são relatados os parâmetros hematológicos, bioquímicos e hormonais em um peixe-boi da Amazônia macho jovem, medidos antes do transporte, imediatamente depois, e durante a sua adaptação a um novo recinto. O animal foi transportado de Manaus, Estado do Amazonas, Brasil, para São Paulo, Estado de São Paulo, Brasil, (2.733 km) durante 6h de viagem. Entre os parâmetros sanguíneos analisados, foi observada clara neutrofilia, linfopenia e aumento na relação neutrófilo/linfócito e nos níveis de glicose e aspartato aminotransferase (AST), mas estes parâmetros posteriormente voltaram aos valores normais. Estes resultados sugerem que o transporte e as mudanças no ambiente são eventos estressantes para o peixe-boi da Amazônia. Desta forma, é recomendável o monitoramento dos parâmetros hematológicos e bioquímicos antes e após a translocação para minimizar os efeitos de agentes estressores nesta espécie.

Palavras-chave: sirênio, hematologia, bioquímica sanguínea, cortisol, estresse.

Introduction

The stress response is an adaptive response essential to survival that is well conserved amongst different species (PIZZUTTO et al., 2009; ST. AUBIN et al., 2013). Stress events stimulate the hypothalamus-pituitary-adrenocortical axis and the sympatho-adrenomedullary system, increasing glucocorticoid and catecholamine secretions that provide protection under stressful conditions (MÖSTL; PALME, 2002). Consequently, the action of these hormones alters the concentration of blood components (JAIN, 1993; MORMEDE et al., 2007).

Transportations and changes in the captivity environment have been reported as temporary stressful situations for various animals (FAZIO; FERLAZZO, 2003; HULBERT et al., 2011; PIZZUTTO et al., 2008, 2009; WESCHENFELDER et al., 2012). The stressful situation may affect immune system function and increase susceptibility to infectious diseases, and, if overactivated, it can be detrimental and even result in death (NODA et al., 2007; PIZZUTTO et al., 2009; ST. AUBIN et al., 2013). Therefore, the stress monitoring is extremely important to minimize its deleterious effects.

The Amazonian manatee (*T. inunguis*, MAMMALIA: SIRENIA) is an endangered aquatic mammal endemic to the Amazon basin, and there is no information concerning the stress responses in this species. Therefore, in this study we report the blood parameters of a captive Amazonian manatee measured before transport, immediately after transport, and during adaptation to a new facility.

Material and methods

A healthy juvenile male Amazonian manatee (8 years old; 108 kg) was transported from the Laboratory of Aquatic Mammals of the National Institute of Amazonian Research LMA/INPA, Manaus, Amazonas State, Brazil, to the São Paulo Aquarium, São Paulo, São Paulo State, Brazil. At LMA/INPA, the manatee was housed in a circular pool (3 m deep x 10 m wide) with 14 other manatees.

The *T. inunguis* was transported at night via cargo truck to Manaus airport (12 km). Subsequently, the animal was transported in a cargo airplane to São Paulo (2,689 km; 3h 30 minutes travel time). In São Paulo, the animal was then transported via cargo truck to the aquarium (32 km). During transport the manatee was kept on a thick mattress covered with a wet towel, and its skin was periodically wetted. The internal airplane temperature was acclimatized to 24°C, and all aerial transport procedures were performed under IATA (International Air Transport Association) regulations and with Brazilian government (IBAMA) authorization. All operations were performed at night, within 6 h, and monitored by a veterinarian.

At the São Paulo Aquarium, the manatee was fed and housed in a handling pool. The next day, the animal was transferred to an exhibition pool (rectangular, 4.9 m deep x 16.25 m long x 9.50 m wide). This pool was environmentally enriched to mimic Amazon rivers (artificial submerged trees and wall paintings). After a four-day adaptation period 49 large Amazonian fishes (arapaima, tambaqui, and others) were introduced into the pool, in small amounts, for 3 consecutive days.

Blood samples were collected from the brachial vascular bundle at 2 weeks before transport, immediately before transport, immediately after arrival in São Paulo, and 2 additional times after the introduction of the fishes (7 and 15 days after transport). The hematological and biochemical parameters were analyzed using automated machines (ABX Pentra 120®, Horiba Medical, France; Architect C800®, Abbott Laboratories, USA) and commercial kits (Labtest Diagnostica S.A., Minas Gerais State, Brazil). The blood smears were stained using the May-Grunwald-Giemsa methods, and the same person manually counted the differential leukocytes (one hundred leukocytes were examined in duplicate). The serum cortisol levels were measured using a commercial radioimmunoassay (RIA) kit (Coat-a-Count, Siemens, Los Angeles, CA, USA) developed for human serum. All results were compared with the normal values previously reported for juvenile male Amazonian manatees (CARMO, unpublished data). The cortisol values were compared only among events because there are no reference values for Amazonian manatees.

Results

Among all parameters analyzed, we observed a clear neutrophilia, lymphopenia, and an increase in the neutrophil/lymphocyte ratio (150%), serum glucose (79%), and aspartate aminotransferase (AST) (300%) levels after transportation (Table 1).

Table 1. The blood parameters of an Amazonian manatee (*Trichechus inunguis*) before transport, immediately after transport, and during adaptation to a new facility.

Parameters	Reference values[a] Mean (range)	Days relative to transport						
		-14	0[b]	Transport	0[c]	Days 4, 5, and 6 – introduction of fishes	7	15
RBC ($10^6 \mu L^{-1}$)	2.3 (1.9 – 2.8)	2.3	2.5		2.0		2.6	2.1
Hb (g dL^{-1})	10.7 (9.4 - 12.2)	10.2	10.6		10.5		12.7	11.1
Ht (%)	32.2 (28.8 – 38.0)	32.0	30.6		30.0		37.0	30.0
MCV (fL)	137.0 (125.3 – 151.0)	138.0	122.9		150.0		142.4	142.9
MCH (pg)	45.6 (42.2 - 51.6)	44.0	42.6		52.5		48.8	52.9
MCHC (g dL^{-1})	33.3 (32.1 - 36.2)	31.9	34.6		35.0		34.3	37.0
WBC ($10^3 \mu L^{-1}$)	10.2 (6.9 - 13.5)	8.6	8.1		7.8		11.0	8.8
NE ($10^3 \mu L^{-1}$)/%	4.8 (3.4 - 6.9)	3.1 / 36%	3.7 / 46%		4.9 / 63%		4.9 / 45%	3.7 / 42%
LY ($10^3 \mu L^{-1}$)/%	4.0 (2.4 - 6.1)	4.6 / 53%	3.7 / 46%		2.2 / 28%		5.2 / 47%	4.0 / 45%
EO ($10^3 \mu L^{-1}$)/%	0.9 (0.6-1.7)	0.6 / 7%	0.3 / 3%		0.4 / 5%		0.6 / 5%	0.6 / 7%
MO ($10^3 \mu L^{-1}$)/%	0.7 (0.3 - 1.2)	0.3 / 4%	0.4 / 5%		0.2 / 3%		0.3 / 3%	0.4 / 5%
BA ($10^3 \mu L^{-1}$)/%	0.03 (0.0 - 0.1)	0 / 0%	0 / 1%		0.1 / 1%		0 / 0%	0.1 / 1%
N:L ratio	0.9 (0.6 - 1.4)	0.7	1.0		2.2		0.9	0.9
Albumin (mg dL^{-1})	4.4 (2.9 - 4.9)	2.5	2.5		3.1		3.1	3.1
Globulin (mg dL^{-1})	2.1 (1.3 - 3.5)	4.2	4.1		3.1		3.7	3.3
Total Protein (g dL^{-1})	6.3 (4.9 - 7.8)	6.7	6.6		6.2		6.8	6.4
Cholesterol (mg dL^{-1})	150.7 (94.0 – 157.0)	159.0	151.0		143.0		167.0	148.0
Triglycerides (mg dL^{-1})	95.0 (59 - 153)	130.0	121.0		114.0		44.0	90.0
ALT (U L^{-1})	11.6 (5.5 – 32.0)	9.0	6.0		6.0		10.0	12.0
AST (U L^{-1})	13.0 (6.0 – 33.0)	8.0	7.0		30.0		25.0	10.0
Urea (mg dL^{-1})	30.7 (19.0 - 40.2)	52.0	43.0		46.0		46.2	47.3
Glucose (mg dL^{-1})	49.9 (35.0 – 66.0)	41.0	38.0		71.0		71.0	47.9
Cortisol (μg dL^{-1})	---	< 0.04	< 0.04		< 0.04		0.4	0.5

RBC: red blood cell; Hb: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; NE: neutrophil; LY: lymphocyte; EO: eosinophil; MO: monocyte; BA: basophil; N:L: neutrophil/lymphocyte ratio; ALT: alanine transaminase; AST: aspartate aminotransferase. [a]Reference values from Carmo (unpublished data). [b]Immediately before transport. [c]Immediately after transport.

These parameters remained high after the introduction of the fishes into the pool; however, all of these parameters returned to normal values at 15 days after transportation (9 days after final fishes introduction). The values for the other parameters were within the normal range, and low variations were considered individual characteristics. The cortisol values remained lower than the sensibility test value (0.04 μg dL^{-1}) before and after transport, which were detected at low values after the introduction of the fishes.

Discussion

Similar alterations in the blood parameters observed in this study have been reported for other aquatic mammals under stressful conditions (CHAMPAGNE et al., 2012; HAO et al., 2009; LANYON et al., 2012; MANIRE et al., 2003; NODA et al., 2007; O'SHEA et al., 1985). Changes in the immune response might reflect numerous stressors, such the increased duration of manatee transport out of the water associated with food deprivation during handling time. Manire et al. (2003) observed reduced lymphocyte proliferative responses when two adults Florida manatees (*T. manatus latirostris*) received only 20% of their normal diet. Similarly, Hao et al. (2009) observed significant neutrophilia in free-ranging male Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*) under the stress of capture and handling conditions, as observed in the present study.

The reduction in the lymphocyte percentage and increase in the neutrophil percentage and N L^{-1} ratio also indicate transportation stress in other mammals (CHACON et al., 2005; KIM et al., 2005; MIRANDA-DE LA LAMA et al., 2011).

According to Jain (1993), glucocorticoids and catecholamines induce transient leukocytosis through the shifting of cells from peripheral into principal vessels.

Stress-related hormones also increase the blood glucose concentration through mobilized energy reserves, such as tissue glycogen through glycogenolysis (MORMEDE et al., 2007), consistent with the rise of glucose levels observed in this study. This hyperglycemia was also reported in cetaceans and pinnipeds under stressful situations (CHAMPAGNE et al., 2012; HAO et al., 2009). Therefore, the intense manipulation, such as transport, of the Amazonian manatee induces stress. Initially, the introduction of the animal to an enriched environment was also stressful, but after the habituation phase, the blood parameters returned to normal levels, suggesting the apparent adaptation to the new environment (PIZZUTTO et al., 2008).

Although glucocorticoids control all stress responses, the cortisol values did not show alterations. According to Tripp et al. (2011), cortisol is the predominant manatee immunoreactive glucocorticoid. However, Manire et al. (2003), Ortiz et al. (1998) and Tripp et al. (2011), evaluating West Indian manatees (*T. m. manatus* and *T. m. latirostris*) under stress conditions, also observed alterations in blood parameters, but not in the cortisol values. These authors observed reduced values for the serum cortisol (range 0.0 - 1.3 μg dL^{-1}), as observed in this study. The immunoassays used to measure the cortisol levels in these studies and the present study, were developed for human serum. Therefore, although cortisol exhibits a stable structure, perhaps the antibodies from these immunoassays do not cross-react with cortisol in manatee serum. Thus, these assays did not demonstrate the hormonal variations in manatees.

Although there was some increase in the AST levels, the values remained within normal parameters. The increase in AST (also known as serum glutamic oxaloacetic transaminase, or SGOT) levels might reflect excessive handling or rigorous exertion in aquatic mammals (LANYON et al., 2012; MANIRE et al., 2003; O'SHEA et al., 1985), consistent with the alterations observed in the present study after extended transportation and fish introduction.

Conclusion

The present study is the first to report the clinic-pathological monitoring of long distance transportation for the Amazonian manatee. These results suggest that transportation and changes in the environment are temporary stressful events; thus, the glucose levels and N L^{-1} ratio are adequate tools for monitoring of this acute stress. We, therefore, recommend monitoring the hematological and biochemical parameters (primarily the N L^{-1} ratio and glucose levels) before and after translocation to minimize the effects of handling stressors, manifested as changes in hormone levels and immune responses.

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