Aquatic Mammals 2023, *49*(5), 443-461, DOI 10.1578/AM.49.5.2023.443

Hematology and Blood Chemistry Reference Intervals for Antillean Manatees (*Trichechus manatus manatus*) in Colombia

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Abstract

Hematology and blood chemistry tests constitute an easy-to-apply veterinary tool that evaluates an organism's systemic functioning and disease process by comparing the level of specific analytes against species norms. Such analyses help monitor marine mammals' health and nutritional status. Although reference values have been published for a few manatee populations, there are none for Antillean manatees (*Trichechus manatus manatus*) in Colombia. We aim to establish the reference values for hematology and serum chemistries for these manatees and determine if there are variations between individuals of different age groups and sex. Thus, we obtained whole blood and serum samples from 45 rehabilitated manatees from Colombia between 1992 and 2021. Complete Blood Count and Comprehensive Metabolic Panel values were calculated, and differences between age groups and sex were determined. Results were compared with published reference intervals of other Antillean manatee populations, Florida manatees (*Trichechus manatus latirostris*), and Amazonian manatees (*Trichechus inunguis*) from Brazil. We determined the reference intervals of hematology and serum chemistry for manatees in Colombia for different age and sex categories. No relevant clinical variations were found in hematological parameters due to sex. Marked differences were found between age groups, mainly among young animals with an expected faster metabolism. There were significant variations between hematological and blood chemistry values when the Colombian manatees were compared to manatees from Puerto Rico and Amazonian manatees from Brazil. Such variations are likely influenced by evolutionary history and environmental factors associated with differences in habitat salinity and diet. We recommend that future studies correlate these blood tests with specific panels. We further recommend conducting wild manatee health assessments as this information will yield essential data for species management schemes needed due to the multiple anthropogenic and environmental threats that manatees face today which put the Colombian manatee's health and ultimate survival at risk.

Key Words: hematology, Complete Blood Count, blood chemistry, Comprehensive Metabolic Panel, Colombia, manatee

Introduction

Antillean manatees (*Trichechus manatus manatus*) are a subspecies of the West Indian manatee (*Trichechus manatus*) (Domning & Hayek, 1986) that inhabit coastal marine, estuarine, and riverine habitats from the southwest Gulf of Mexico, throughout the Greater Antilles and Caribbean coasts of Central and South America, to the Atlantic coasts of the Guyanas and Brazil (Self-Sullivan & Mignucci-Giannoni, 2012). Due to

multiple environmental and anthropogenic threats and low population density, these herbivorous aquatic mammals are listed as "Endangered" by the International Union for the Conservation of Nature (IUCN) (Morales-Vela et al., 2024).

Part of the recovery efforts for the species includes rescue and rehabilitation programs in key countries such as Belize, Brazil, Colombia, Mexico, Puerto Rico, and the United States (Adimey et al., 2012), and animals maintained under human care in the Caribbean, South America, Europe, and Asia (von Fersen, 2019). Veterinary treatment and long-term care of this species require diagnostic tools that can be used to monitor their rehabilitation process and health and, thus, inform caregivers of the animals' status to prepare them for release or ensure continued maintenance under human care. Among these diagnostic aids, reference values for hematological and serum biochemical analyses are one of the tools most used by veterinarians; they allow for an objective diagnostic approach to evaluate the status of the systemic functionality of an individual and facilitate the prevention, diagnosis, and control of diseases quickly and efficiently (Maceda-Veiga et al., 2015).

Hematological and serum biochemical values have been reported extensively for the Florida manatee subspecies (*Trichechus manatus latirostris*) (Bossart et al., 2001; Manire et al., 2003; Harr et al., 2006, 2008, 2011; Harvey et al., 2007, 2009, 2018, 2019; Ball, 2020, 2021). Similar values for Antillean manatees have been reported for animals in Belize (Sulzner et al., 2012; Siegal-Willott et al., 2013), Brazil (Silva et al., 2007, 2009; Mendonça et al., 2020), Guyana (Converse et al., 1994), Mexico (Olivera-Gómez et al., 2011; Melesio-Navarro, 2014), and Puerto Rico (Jiménez-Marrero et al., 1998; Mignucci-Giannoni & Alsina-Guerrero, 2022). However, conceptually, diagnosis using blood reference values may differ between different species, subspecies, or even individuals within a population due to differences in anatomy, physiology, osmolarity, age, sex, and the environment where they live—whether marine, estuarine, or riverine (Ortíz, 2001; Harvey et al., 2007).

We aim to establish the reference values for the hematology and serum chemistry of the Colombian manatee population and determine, as has been shown in other manatee populations and other wildlife, if there are variations between individuals of different age groups, sex, and habitats.

Methods

Animals

We collected blood samples from 45 rescued manatees from 1992 to 2021 from Córdoba, Bolívar, Atlántico, Sucre, and Magdalena departments (states) in Colombia (Table 1; Figure 1). Manatees were caught in nets deployed from shore (Millán-Sánchez, 1999; Montoya et al., 2001; Caicedo-Herrera et al., 2013) or captured by hand in the case of orphaned calves. The captured manatees were immediately transported to a shaded area where they were kept moist with water buckets and wet towels throughout the health assessment. Data collected included sex, complete body measurements, tissue samples for genetic analysis, and blood drawn by one of the listed authors of the present article. In addition, an experienced manatee veterinarian or veterinary technician, with the aid of marine biologists, conducted a complete physical veterinary examination, including visual external and behavioral assessment, heart rate, respiratory rate, and other vital signs (Millán-Sánchez, 1999; Wong et al., 2012; Mignucci-Giannoni et al., 2015). Manatees were categorized into three age classes based on total length (straight length: tip of snout to notch of fluke) as in Mignucci-Giannoni et al. (2000) or from known ages: calves (< 2 y old; < 175 cm), subadults (2 to 7 y old; 176 to 225 cm), and adults (> 7 y old; > 225 cm). Upon data and sample collection, the manatees were immediately returned to the water at their capture site, with some fitted with a radio-transmitter for tracking studies and released to the wild (Caicedo-Herrera et al., 2013) or, in the case of orphaned calves, maintained in rehabilitation until completion of the time prescribed for their rehabilitation process and then released.

Blood Collection and Sample Preparation

Blood samples were obtained by venipuncture from the medial interosseous space of the radius and ulna, constituting the brachial vascular bundle (Figure 2). Before collection, the pectoral flipper was surgically scrubbed with povidone-iodine and isopropyl alcohol, or post-2017, with chlorhexidine scrub, chlorhexidine solution, and isopropyl alcohol. Blood was obtained using an 18- to 21-gauge, ¾ to 1½" needle with an attached "butterfly" BD vacutainer blood collection set (Becton, Dickinson & Company, Franklin Lakes, NJ, USA) or 14" extension set (International WIN, Limited, Kenneth Square, PA, USA), depending on the size of the manatee. Blood was collected first for serum chemistry analysis directly into 6- to 10-ml red top sterile vacutainer tubes with a silicone-coated interior and

Table 1. Antillean manatees (*Trichechus manatus manatus*) sampled in Colombia for this study between 1992 and 2021; A = adult (A), SA = subadult, and C = dependent calf.

Date	Field number	Name	Sex	Length (cm)	Weight (kg)	Age class	Location
22 June 1992	NEPST151	Sayida	F	229	185	A	Bocas de Ceniza, Atlántico
29 June 1992	NEPST213	Pluto	M	218	165	SA	Brazo Loba, Bolivar
29 June 1992	NEPST220	Lulu	F	201	98	SA	Brazo Loba, Bolivar
29 June 1992	NEPST221	Jhoni	M	211	169	SA	Las Brisas, Bolivar
30 June 1992	NEPST155	Irotama	F	218	166	SA	Brazo Loba, Bolivar
30 June 1992	NEPST156	Luz	F	302	450	A	Boca Rio Sinú, Cordoba
30 June 1992	NEPST157	Panorama	F	238	220	A	Tacaloa, Bolivar
30 June 1992	NEPST217	Rick II	M	129	31	C	Magangué, Bolivar
30 June 1992	NEPST218	Anapoima	F	241	205	A	Brazo Loba, Bolivar
1 July 1992	NEPST153	Chimiquique	M	242	212	A	Brazo Mompox, Bolivar
1 July 1992	NEPST154	Limonar	M	215	137	SA	Brazo Loba, Bolivar
1 July 1992	NEPST166	Manantial	M	210	--	SA	Brazo Loba, Bolivar
1 July 1992	NEPST216	Roma	F	163	95	C	Tacamocho, Bolivar
1 July 1992	NEPST219	Cañaguate	M	252	169	A	Talaigua, Bolivar
2 July 1992	NEPST214	NEPST214	F	203	--	SA	El Retiro, Bolivar
2 July 1992	NEPST215	NEPST215	M	210	--	SA	El Retiro, Bolivar
7 July 1992	NEPST224	MacGyver	M	192	39	SA	Lorica, Córdoba
7 July 1992	NEPST226	Hmno McGyver	M	208	--	SA	Río Sinú, Córdoba
12 July 1995	NEPST410	NEPST410	M	187	107	SA	Brazo Loba, Bolivar
13 Nov 2009	COLTm0801	Julieta I	F	243	232	A	Lorica, Córdoba
14 Nov 2009	NEPST225	Juana	F	315	484	A	Lorica, Córdoba
13 Nov 2010	COLTm0803	Romeo	M	219	189	SA	Lorica, Córdoba
7 Nov 2011	CO2011583	David	M	228	163	A	Lorica, Córdoba
17 Nov 2011	NEPST227	Chiqui	F	308	423	A	Cienaga Ayapel, Córdoba
17 Nov 2011	NEPST228	Ruby	F	321	500	A	San Bernardo del Viento, Córdoba
17 Nov 2011	--	Maria del Mar	F	197	89	SA	La Balsa, San Bernardo del Viento, Córdoba
22 July 2012	--	María Fe	F	200	94	SA	Finca Santa Fé, Bolivar
17 Nov 2012	CO2011550	Angélica	F	248	206	A	Lorica, Córdoba
21 June 2013	--	Willy	M	299	341	A	El Castillo, San Bernardo del Viento, Córdoba
4 June 2014	--	Batata	F	234	166	A	Mahates, Bolivar
9 June 2014	--	Carolina	F	186	108	SA	Mahates, Bolivar
26 Aug 2014	--	Jonas	M	131	25	C	Tacamocho, Bolivar
21 Oct 2014	--	Gleimer	M	233	181	A	Mahates, Bolivar
27 Nov 2015	--	Margarita	F	242	149	A	Cispatá, Córdoba
20 April 2016	-	Tico	M	221	159	SA	Mahates, Bolivar
28 April 2016	--	Jaraba	M	298	310	A	Cienaga de Jaraba, Magdalena
18 Nov 2019	--	Santiago	M	233	180	A	Santiago de Tolu, Sucre
21 Nov 2019	--	Esperanza	F	206	135	SA	Mahates, Bolivar
21 Nov 2019	--	Hugo	M	223	180	SA	Mahates, Bolivar
21 Nov 2019	--	Isabel	F	205	123	SA	Mahates, Bolivar
21 Nov 2019	--	Jey-Jey	M	220	145	SA	Mahates, Bolivar
21 Nov 2019	--	Lila	F	219	142	SA	Mahates, Bolivar
21 Nov 2019	--	Sebastian	M	209	150	SA	Mahates, Bolivar
12 June 2021	FO210603Tm01	Julieta II	F	322	442	A	Isla de la Aguja, Magdalena
1 Sept 2021	FO210901Tm01	Jerito	M	132	33	C	Tasajeras, Magdalena

Figure 1. Collection locations (yellow squares) for Antillean manatees (*Trichechus manatus manatus*) included in this study

no additives (Becton, Dickinson & Company), allowed to clot in a cool and shaded area, and then separated by centrifugation (3,200 rpm × 15 min) using an Adams Compact II Centrifuge (Becton Dickinson & Company, Sparks, MD, USA). Next, the separated serum was collected into cryogenic tubes for storage and analyses (Brooks et al., 2022). Following this, blood was collected for a Complete Blood Count (CBC) analysis into 3- to 4-ml lavender top sterile vacutainer tubes, which contained EDTA (K2EDTA; Becton, Dickinson & Company) as the anticoagulant, agitated gently, and kept cool until analyzed. Both serum and whole blood were refrigerated in ice and transported to a clinical reference laboratory for processing.

Complete Blood Count (CBC)

The CBC provided values for white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HBG), hematocrit (HCT), platelet (PLTS), and the red blood indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and red cell distribution width (RDW). CBC analysis was conducted with a BC6800 Auto Hematology Analyzer (Mindray Bio-Medical Electronics, Shenzhen, Guangdong, China). In addition, a manual leukocyte differential was conducted under the microscope to allow for the evaluation of different leukocyte cell types, identifying and enumerating lymphocytes (LYMP), monocytes (MONO), eosinophils (EOSI), basophils (BASO), and heterophils (HETE) in percentage values. In automated CBC machines, heterophils are usually wrongly categorized as eosinophils due to their similarity in granulation morphology. In manatees, as in elephants, hyraxes, and rabbits, their poly-morphonuclear leukocytes (PMNs) are heterophilic-like cells and not neutrophilic as in other mammals (Bossart & Dierauf, 1990). This study used medical technologists and veterinarians who were specifically trained in manatee leukocyte identification and manual counting for correct categorization and counting.

Serum Chemistry

We performed a Comprehensive Metabolic Panel (CMP) on all the serum samples with an AU400

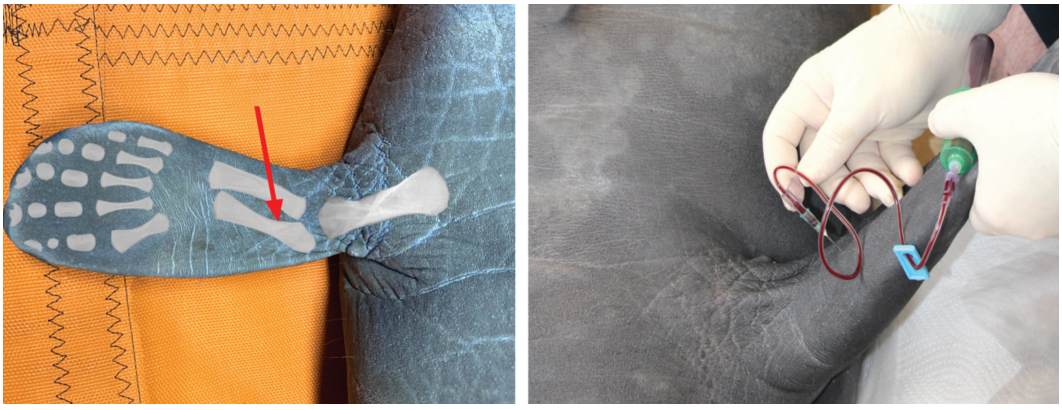

Figure 2. Location for venipuncture between the radius and ulna of the palmar section of the flipper of an Antillean manatee

Chemistry Analyzer (Olympus and Beckman Coulter, Breinigsville, PA, USA). The resulting chemistry analytes were grouped based on six physiological processes: (1) liver-associated enzymes and pigments (lactate dehydrogenase [LDH], direct bilirubin [DIR BILI], indirect bilirubin [IND BILI], total bilirubin [TOT BIL]); (2) muscle-associated enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], creatine phosphokinase [CPK]); (3) kidney-associated compounds and products (blood urea nitrogen [BUN], creatinine [CREA], blood urea nitrogen:creatinine ratio [BUN:CREA], uric acid [UA]); (4) sugars, lipids, and pancreatic-associated enzymes (glucose [GLU], triglycerides [TRIG], cholesterol [CHOL], amylase [AMY]); (5) proteins (total protein [TOT PROT], albumin [ALB], globulin [GLOB], albumin:globulin ratio [ALB:GLOB]); and (6) electrolytes (sodium [Na]; chloride [Cl^-]; potassium [K]; phosphate [PO_4], sometimes referred to as phosphorus [P]; calcium [Ca]; magnesium [Mg]).

Statistical Analysis

Microsoft Excel for Mac, Version 12.2.8 (Microsoft Corp., Redmond, WA, USA), was used for initial descriptive analyses. Descriptive statistics (sample size, mean, maximum value, minimum value, and standard deviation) of hematology and serum chemistry values were calculated on all the samples for every parameter. The *Sigma* statistical program was used to determine the normal ranges for each parameter based upon the calculated mean ($\bar{X}$) and standard deviation (SD). First, minimum and maximum intervals defined as ± 2 SD around the mean were calculated, and values outside this range were considered outliers and eliminated. Following this, means and SDs were recalculated, and new reference ranges were determined as ± 1 SD around the mean. Finally, similar to other studies (Silva et al., 2009; Pantoja et al., 2010; Sulzner et al., 2012), an unpaired two-sample *t* test was done comparing males vs females and adults vs subadults to determine any significant differences ($p \leq 0.05$) between each of the parameters. Although ideally the sample size is preferred to be larger, the challenges of working with an endangered species can make this difficult. Nevertheless, we have been able to use sample sizes similar to those presented in other manatee studies (e.g., Silva et al., 2009; Sulzner et al., 2012). To avoid a Type I Error and to corroborate our findings, we performed a Bonferroni Correction Post Hoc Test. Calves were not included in the latter due to the small sample size. This test was also used to identify if there were significant differences between the Colombian population and other manatee populations (Puerto Rico's Antillean manatee and Brazil's Amazonian manatee [*Trichechus inunguis*]) based on raw data reported in the literature.

Results

Forty-five rescued manatees from Colombia were included in the study after they were deemed clinically healthy or during a pre-release checkup (Table 1). Of these, 23 were males, 22 were females, four were calves, 22 were subadults, and 19 were adults. Sex by age group was not evenly distributed, with three male calves and one female calf, 13 male and nine female subadults, and seven male and 12 female adults. The geographic distribution was also not even, with most animals sampled around Magangué in Bolivar and Lorica and the Sinú River in Córdoba (Figure 1).

Hematological parameters were obtained for the study group, and results were presented as

Table 2. Mean and standard deviation (SD) of hematology values for Antillean manatees from Colombia for all sex classes (males and females, $n = 45$; males only, $n = 23$; and females only, $n = 22$), with ± 1 SD and ranges in parentheses. Statistical significance based on the Bonferroni Correction was $p = 0.0026$.

	All manatee samples		Males		Females		
Parameter	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	*p* values
WBC (10^9/L)	5.2 ± 1.9	(3.3-7.1)	5.0 ± 2.4	(2.6-7.4)	5.3 ± 1.5	(3.9-6.8)	0.70
RBC (10^6/mm^3)	3.1 ± 0.4	(2.7-3.6)	3.3 ± 0.3	(3.0-3.6)	3.0 ± 0.5	(2.5-3.5)	0.07
HGB (g/dL)	10.7 ± 0.9	(9.8-12)	11.0 ± 1.0	(10-12)	10.5 ± 0.9	(9.6-11)	0.20
HCT (%)	32.7 ± 2.8	(30-36)	33.7 ± 3.3	(30-37)	31.8 ± 2.0	(30-34)	0.10
PLTS (10^3/mm^3)	325.5 ± 102.3	(223-428)	302.1 ± 87.7	(214-390)	349.0 ± 114.3	(235-463)	0.29
Red blood cell indices							
MCV (fL)	105.0 ± 15.3	(90-120)	102.5 ± 13.8	(89-116)	107.1 ± 16.8	(90-124)	0.48
MCH (pg)	34.0 ± 3.9	(30-38)	33.6 ± 3.4	(30-37)	34.3 ± 4.4	(30-39)	0.69
MCHC (g/dL)	32.3 ± 1.3	(31-34)	32.6 ± 0.8	(32-33)	32.0 ± 1.5	(30-34)	0.21
MPV (fL)	6.0 ± 0.6	(5.5-6.6)	6.1 ± 0.6	(5.5-6.7)	6.0 ± 0.6	(5.4-6.6)	0.86
RDW (%)	15.7 ± 1.0	(15-17)	15.2 ± 0.9	(14-16)	15.9 ± 1.0	(15-17)	0.31
White blood cell differential							
LYMP (%)	29.5 ± 14.1	(15-44)	30.2 ± 15.8	(14-46)	28.6 ± 12.2	(16-41)	0.73
MONO (%)	2.7 ± 2.8	(0-6)	2.5 ± 3.1	(0-6)	2.9 ± 2.5	(0-6)	0.61
EOSI (%)	0.6 ± 1.1	(0-2)	1.0 ± 1.3	(0-2)	0.3 ± 0.6	(0-1)	0.04
BASO (%)	0.2 ± 0.6	(0-1)	0.2 ± 0.4	(0-1)	0.3 ± 0.9	(0-1)	0.49
HETE (%)	67.0 ± 15.1	(52-82)	66.3 ± 16.6	(50-83)	67.9 ± 13.5	(55-81)	0.75

Note: WBC = white blood cell count, RBC = red blood cell count, HBG = hemoglobin, HCT = hematocrit, PLTS = platelet count, MCV WBC = white blood cell count, RBC = red blood cell count, HBG = hemoglobin, HCT = hematocrit, PLTS = platelet count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MPV = mean platelet volume, RDW = red cell distribution width, LYMP = lymphocytes, MONO = monocytes, EOSI = eosinophils, BASO = basophils, and HETE = heterophils

averages between males and females (Table 2), and subadults and adults (Table 3). Leukocytes in the Colombian manatee population were composed primarily of heterophils (67.0%; range of 52 to 82%) and lymphocytes (29.5%; range of 15 to 44%), with few monocytes, and rare eosinophils and basophils. There were no observed significant differences between males and females or between subadults and adults regarding hematological values. When comparing the mean values and deviations of the Colombian to the Puerto Rican populations (Mignucci-Giannoni & Alsina-Guerrero, 2022), significant differences were detected among some hematological values, including red blood cell count ($p = 0.0031$), hemoglobin ($p = 0.0233$), mean corpuscular volume ($p = 0.0001$), mean corpuscular hemoglobin ($p = 0.0001$), red cell distribution width ($p = 0.013$), lymphocytes ($p = 0.0001$), and heterophils ($p = 0.0001$) (Table 4).

Serum chemistry parameters were also obtained for the entire population sampled, and were averaged across males and females (Table 5), and subadults and adults (Table 6). Triglycerides ($p = 0.0003$) were greater in subadults vs adults, but no other variables were different based on sex or age class (Tables 5 & 6). With few exceptions (aspartate aminotransferase, creatine phosphokinase, sodium, and potassium), all of the mean serum biochemistries were significantly different between the Colombian and Puerto Rican manatees (Mignucci-Giannoni & Alsina-Guerrero, 2022; Table 7). When the Colombian Antillean manatee and the freshwater Amazonian manatee (de Mello et al., 2011; Maduro et al., 2020) populations were compared, significant differences in all kidney-associated compounds, products, and proteins were found. Within the electrolytes, all but sodium were statistically different. In the sugars, lipids, and pancreatic-associated enzymes, all were statistically different except for glucose and cholesterol. There were no significant differences in the liver-associated enzymes and pigments or the muscle-associated enzymes.

Table 3. Mean and SD hematology values for Antillean manatees from Colombia for all age classes (calves, subadults, and adults, $n = 45$; subadults only, $n = 22$; and adults only, $n = 19$), with ± 1 SD and ranges in parentheses. Statistical significance based on the Bonferroni Correction was $p = 0.0026$.

	All manatee samples		Subadults		Adults		
Parameter	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	*p* value
WBC (10^9/L)	5.2 ± 1.9	(3.3-7.1)	4.3 ± 1.1	(3.2-5.4)	5.8 ± 2.2	(3.6-8.0)	0.048
RBC (10^6/mm^3)	3.1 ± 0.4	(2.7-3.6)	3.2 ± 0.5	(2.8-3.7)	3.0 ± 0.4	(2.6-3.5)	0.30
HGB (g/dL)	10.7 ± 0.9	(9.8-12)	10.5 ± 0.9	(9.6-11)	10.6 ± 0.8	(9.8-12)	0.65
HCT (%)	32.7 ± 2.8	(30-36)	31.9 ± 2.0	(30-34)	32.5 ± 2.6	(30-35)	0.57
PLTS (10^3/mm^3)	325.5 ± 102.3	(223-428)	281.4 ± 59.0	(222-340)	354 ± 123.7	(230-478)	0.12
Red blood cell indices							
MCV (fL)	105.0 ± 15.3	(90-120)	98.0 ± 11.7	(86-110)	108.1 ± 16.6	(92-125)	0.11
MCH (pg)	34.0 ± 3.9	(30-38)	32.6 ± 3.3	(29-36)	34.4 ± 4.1	(30-39)	0.27
MCHC (g/dL)	32.3 ± 1.3	(31-34)	32.6 ± 1.0	(32-34)	31.8 ± 1.4	(30-33)	0.06
MPV (fL)	6.0 ± 0.6	(5.5-6.6)	5.7 ± 0.4	(5.3-6.1)	6.2 ± 0.6	(5.6-6.8)	0.23
RDW (%)	15.7 ± 1.0	(15-17)	16.4 ± 1.0	(15-17)	15.1 ± 0.6	(15-16)	0.08
White blood cell differential							
LYMP (%)	29.5 ± 14.1	(15-44)	28.9 ± 14.1	(15-43)	32 ± 13.9	(18-46)	0.54
MONO (%)	2.7 ± 2.8	(0-6)	3.4 ± 3.4	(0-7)	2.2 ± 1.9	(0-4)	0.25
EOSI (%)	0.6 ± 1.1	(0-2)	1.0 ± 1.2	(0-2)	0.4 ± 0.9	(0-1)	0.13
BASO (%)	0.2 ± 0.6	(0-1)	0.1 ± 0.3	(0-1)	0.4 ± 0.9	(0-1)	0.20
HETE (%)	67.0 ± 15.1	(52-82)	66.6 ± 15.3	(51-82)	65.1 ± 14.7	(50-80)	0.78

Note: WBC = white blood cell count, RBC = red blood cell count, HBG = hemoglobin, HCT = hematocrit, PLTS = platelet count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MPV = mean platelet volume, RDW = red cell distribution width, LYMP = lymphocytes, MONO = monocytes, EOSI = eosinophils, BASO = basophils, and HETE = heterophils

Discussion

Blood tests provide a diagnostic approach that can correlate the individual's health and systemic functionality with the influence of environmental factors, allowing for the prevention and control of disease quickly and effectively (Millán-Sánchez, 1999; Maceda-Veiga et al., 2015). Early evaluation and detection of diseases can reduce costs associated with treating diseases or conditions, long-term care of the animals, and preventing recurrences due to inadequate treatment or diagnosis. Thus, routine hematology and blood chemistry analysis become critical in conserving and caring for endangered species such as the manatee (Harvey et al., 2009; Silva et al., 2009).

Complete Blood Count

Erythrocyte parameters, red blood cell indices, leukocytes, and differential count in Colombian manatees were generally similar to those reported for other Antillean and Florida manatee populations. The significant differences between Puerto Rican and Colombian populations were probably determined by factors such as age and environmental conditions (Silva et al., 2007; Sulzner et al., 2012; Mendonça et al., 2020). Therefore, the defined ranges will help establish values for veterinary diagnostic purposes specifically to assess an individual's health status within the population (Brooks et al., 2022).

The red cell distribution width (RDW) found in Colombia and Puerto Rico was lower than that reported in wild manatees and those under human care in Florida. However, these lower values are considered to lack clinical importance (Hooijberg, 2022). Usually, these morphological changes may indicate deficiency anemia due to iron or B complex deficit as has been previously reported in horses, humans, and other domestic animals (Melo et al., 2012; Ramires et al., 2019), and recently observed in manatees with malnutrition, nutrient-poor diets, and inflammatory processes (Siegal-Willott et al., 2013; Harvey et al., 2019). In fact, due to this type of deficit, manatees naturally carry out nutrient recycling (B complex) by consuming their feces through coprophagy (Marshall, 1997). Such findings should always be corroborated by

Table 4. Hematology ranges for Antillean manatees from Colombia, Puerto Rico, Guyana, Mexico, Belize, and Brazil, and for Florida manatees (*Trichechus manatus latirostris*). Colombia values included all samples (calves, subadults, and adults). Abbreviations for parameters are detailed in the "Methods" section. Statistical significance based on the Bonferroni Correction was $p = 0.0026$. Significant differences using p values between Colombia and Puerto Rico are indicated with an asterisk (*). Columns with a silcrow (§) signify that the range values are minimum and maximum.

	Antillean manatees						Florida manatees	
Parameter	Colombia $n = 45$	Puerto Rico[a] $n = 70$	Guyana[b] $n = 11$	Mexico[c] $n = 18$	Belize[d] $n = 82$	Brazil[e§] $n = 30$	Florida[f] $n = 30$	Florida[g§] $n = 52$
WBC (10^9/L)	3.3-7.1	4.0-8.0	4.6-8.6	3.9-9.1	3.4-7.9	4.4-11	4.5-13	2.8-14
RBC (10^6/mm^3)	2.7-3.6	2.1-3.3*	2.2-2.8	2.3-3.3	2.2-3.0	2.5-3.0	2.3-3.8	2.2-3.4
HGB (g/dl)	9.8-12	9.0-14*	8.9-11	9.8-13	9.5-11	9.1-11	9.9-13	9.4-14
HCT (%)	30-36	28-41	27-34	31-42	30-37	29-34	30-40	29-44
PLTS (10^3/mm^3)	223-428	207-390	--	138-266	156-385	--	--	111-424
Red blood cell indices								
MCV (fL)	90-120	120-137*	--	--	101-146	109-116	115-139	114-140
MCH (pg)	30-38	40-43*	--	--	36-44	33-38	36-45	37-45
MCHC (g/dL)	31-34	31-34	--	30-33	29-35	29-33	29-33	28-35
MPV(fL)	5.5-6.6	--	--	--	--	--	--	--
RDW (%)	15-17	15-21*	--	--	--	--	--	14-23
White blood cell differential								
LYMP (%)	15-44	28-57*	--	--	--	--	16-46	--
MONO (%)	0-6	1-6	--	--	--	--	0-3	--
EOSI (%)	0-2	0-3	--	--	--	--	1-6	--
BASO (%)	0-1	0-1	0-0	--	--	0-0	0-0	--
HETE (%)	52-82	38-67*	--	--	--	--	30-84	--

[a]Mignucci-Giannoni & Alsina-Guerrero, 2022; [b]Converse et al., 1994; [c]Olivera-Gómez et al., 2011; [d]Sulzner et al., 2012; [e]Mendonça et al., 2020; [f]Bossart et al., 2001; [g]Harvey et al., 2009

measuring erythrocyte indices, the serum iron profile, serum minerals, and the acute-phase protein panel (Rosas et al., 1999; Harr et al., 2011; Harvey et al., 2018).

In Colombia, we found a marginally significant difference between the white blood cell count (WBC) values between adult and subadult manatees. This finding is expected because the WBC and its differential are usually higher in younger animals going through an immune system maturation process; and in the absence of infection, levels decrease towards adulthood (Bossart et al., 2001; Nilsson et al., 2014; Ramires et al., 2019). Compared to females, the apparent eosinophilia in males lacks clinical importance as its presence alone, in the absence of active parasitism, allergies, or basophilia, does not reflect an evident alteration in the organism (Thrall et al., 2022).

Similarly, gross differences were observed in the WBC between Puerto Rican and Colombian animals. Possibly, this may be associated with biological processes (estrous cycles) and age since no signs of infection and other types of changes were observed in the rest of the hematology analytes (Willard & Tvedten, 2011; Sulzner et al., 2012; Cabrias-Contreras et al., 2021). Higher leukocyte values in manatees in association with alterations of other analytes in the leukogram and with higher-than-normal body temperatures (29.5° to 35.8°C; Wong et al., 2012; Martony et al., 2020) appear not to be a good indicator of an inflammatory process but, rather, an indicator of an infectious process. Consequently, monitoring relative changes in the WBC of manatees may be more valuable than relying on total counts for diagnostic purposes (Bossart et al., 2001). To confirm inflammatory processes in this species, it is necessary to obtain full sets of phase protein panels (serum amyloid A [SAA], total proteins, albumin, globulin, and albumin:globulin ratio; Harvey et al., 2019). While for confirming infectious processes, we must correlate leukocytes with the rest of the leukogram analytes, and with immunoglobulin profiles (Jiménez-Marrero et al., 1998); bacterial cultures in feces, urine, and blood (Silva et al., 2017); and procalcitonin levels.

Table 5. Mean and SD hematology values for Antillean manatees from Colombia for all sex classes (males and females, n = 45; males only, n = 23; and females only, n = 22), with ± 1 SD and ranges in parentheses. Abbreviations for parameters are detailed in the "Methods" section.

	All manatee samples		Males		Females		
Parameter	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	p values
Liver-associated enzymes and pigments							
LDH (U/L)	187.4 ± 65.0	(122-252)	205.8 ± 70.6	(135-277)	164.9 ± 52.6	(112-218)	0.16
DIR BILI (mg/dL)	0.13 ± 0.12	(0-0.3)	0.14 ± 0.14	(0-0.3)	0.12 ± 0.11	(0-0.2)	0.50
IND BILI (mg/dL)	0.26 ± 0.14	(0.1-0.4)	0.28 ± 014	(0.1-0.4)	0.24 ± 0.13	(0.1-0.4)	0.35
TOT BILI (mg/dL)	0.37 ± 0.24	(0.1-0.6)	0.41 ± 0.26	(0.1-0.7)	0.34 ± 0.22	(0.1-0.6)	0.57
Muscle-associated enzymes							
ALT (U/L)	26.3 ± 13.2	(13-40)	24.3 ± 13.1	(11-37)	28.6 ± 13.4	(15-42)	0.34
AST (U/L)	10.3 ± 3.3	(7.1-14)	9.8 ± 3.2	(6.6-13)	10.9 ± 3.4	(7.6-14)	0.32
ALP (U/L)	125.9 ± 51.1	(75-177)	122.3 ± 59.1	(63-181)	129.3 ± 43.6	(86-173)	0.68
CPK (U/L)	119.4 ± 86.1	(33-206)	118.2 ± 83.2	(35-201)	120.7 ± 91.4	(29-212)	0.93
Kidney-associated compounds and products							
BUN (mg/dL)	9.6 ± 4.1	(5.5-14)	8.8 ± 3.3	(5.4-12)	10.4 ± 4.7	(5.7-15)	0.22
CREA (mg/dL)	1.8 ± 0.5	(1.3-2.3)	1.8 ± 0.5	(1.3-2.3)	1.8 ± 0.5	(1.2-2.3)	0.96
BUN:CREA	5.2 ± 2.2	(3.0-7.4)	5.2 ± 2.3	(2.8-7.5)	5.3 ± 2.0	(3.3-7.3)	0.88
UA (mg/dL)	1.4 ± 0.9	(0.5-2.2)	1.4 ± 0.9	(0.6-2.3)	1.3 ± 0.9	(0.5-2.2)	0.83
Sugars, lipids, and pancreatic-associated enzymes							
GLU (mg/dL)	73.5 ± 20.9	(53-94)	72.2 ± 18.5	(54-91)	74.9 ± 23.5	(51-99)	0.70
TRIG (mg/dL)	93.3 ± 38.3	(55-132)	97.2 ± 43.0	(54-140)	89.2 ± 33.5	(56-123)	0.54
CHOL (mg/dL)	180.2 ± 40.9	(139-221)	187.8 ± 46.0	(142-234)	172.5 ± 34.6	(138-207)	0.27
AMY (U/L)	442.2 ± 124.3	(318-567)	485 ± 130.2	(355-615)	403.3 ± 110.2	(293-513)	0.14
Proteins							
TOT PROT (g/dL)	6.3 ± 0.4	(5.9-6.7)	6.4 ± 0.4	(5.9-6.8)	6.2 ± 0.4	(5.9-6.6)	0.37
ALB (g/dL)	4.1 ± 0.4	(3.7-4.4)	4.0 ± 0.4	(3.6-4.4)	4.1 ± 0.4	(3.7-4.5)	0.45
GLOB (g/dL)	2.2 ± 0.5	(1.8-2.7)	2.3 ± 0.4	(1.9-2.7)	2.2 ± 0.5	(1.7-2.7)	0.44
ALB:GLOB	1.8 ± 0.4	(1.4-2.3)	1.7 ± 0.3	(1.4-2.1)	1.9 ± 0.5	(1.4-2.5)	0.26
Electrolytes							
Na (mmol/L)	150.8 ± 6.6	(144-157)	150.6 ± 6.7	(144-157)	151 ± 6.6	(144-158)	0.88
Cl^- (mmol/L)	96.0 ± 6.6	(89-103)	96.6 ± 7.0	(90-104)	95.3 ± 6.4	(89-102)	0.54
K (mmol/L)	5.1 ± 0.8	(4.3-5.9)	5.2 ± 0.7	(4.5-5.9)	5.0 ± 0.9	(4.2-5.9)	0.51
PO_4 (mg/dL)	4.2 ± 0.9	(3.2-5.1)	4.1 ± 0.7	(3.4-4.9)	4.2 ± 1.1	(3.0-5.3)	0.90
Ca (mg/dL)	9.3 ± 1.2	(8.1-10)	9.4 ± 1.0	(8.3-10)	9.1 ± 1.3	(7.9-10)	0.54
Mg (mg/dL)	3.4 ± 1.0	(2.4-4.4)	3.5 ± 0.5	(3.0-4.0)	3.4 ± 1.2	(2.2-4.6)	0.91

Serum Chemistry

Liver-Associated Enzymes and Pigments—In the liver-associated panel, we observed that lactate dehydrogenase levels coincide with those reported in different Caribbean and Floridian populations. Despite this, we found that lactate dehydrogenase values in the Colombian population were below the ranges obtained for the same subspecies in Puerto Rico and for the Amazonian species (Table 7). This decrease could be related to a physiological response to increased physical activity while capturing and obtaining the sample. Similar findings have been previously described for horses (Guerrero Nieto et al., 2009). Davis & Walsh (2018) suggested that lactate dehydrogenase is helpful in the clinical assessment of manatees, specifically in cases of watercraft collisions or gastrointestinal disorders. However, it

Table 6. Mean serum chemistry values for manatees from Colombia for all manatees sampled (calves, subadults, and adults, $n = 45$; subadults only, $n = 22$; and adults only, $n = 19$), with ± 1 SD and range in parentheses. Abbreviations for parameters are detailed in the "Methods" section. Statistical significance based on the Bonferroni Correction was $p = 0.0026$. Significant differences using p values between subadults and adults are indicated with an asterisk (*).

	All manatee samples		Subadults		Adults		
Parameter	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	*p* value
Liver-associated enzymes and pigments							
LDH (U/L)	187.4 ± 65.0	(122-252)	177.6 ± 52.6	(125-230)	182.9 ± 71.0	(112-254)	0.86
DIR BILI (mg/dL)	0.13 ± 0.12	(0-0.3)	0.15 ± 0.14	(0-0.3)	0.09 ± 0.09	(0-0.2)	0.16
IND BILI (mg/dL)	0.26 ± 0.14	(0.1-0.4)	0.28 ± 0.13	(0.2-0.4)	0.20 ± 0.12	(0.1-0.3)	0.07
TOT BILI (mg/dL)	0.37 ± 0.24	(0.1-0.6)	0.4 ± 0.24	(0.2-0.6)	0.29 ± 0.19	(0.1-0.5)	0.15
Muscle-associated enzymes							
ALT (U/L)	26.3 ± 13.2	(13-40)	31.4 ± 12.9	(19-44)	20.9 ± 11.5	(9.4-33)	0.02
AST (U/L)	10.3 ± 3.3	(7.1-14)	10.5 ± 3.2	(7.3-14)	9.9 ± 3.6	(6.3-14)	0.65
ALP (U/L)	125.9 ± 51.1	(75-177)	132.2 ± 51.3	(81-184)	119.1 ± 46.1	(73-165)	0.42
CPK (U/L)	119.4 ± 86.1	(33-206)	102.6 ± 77.0	(26-180)	136.8 ± 99.7	(37-237)	0.28
Kidney-associated compounds and products							
BUN (mg/dL)	9.6 ± 4.1	(5.5-14)	8.4 ± 3.4	(5.1-12)	10.9 ± 4.3	(6.6-15)	0.07
CREA (mg/dL)	1.8 ± 0.5	(1.3-2.3)	1.7 ± 0.5	(1.2-2.3)	2.0 ± 0.4	(1.6-2.3)	0.15
BUN:CREA	5.2 ± 2.2	(3.0- 7.4)	5.3 ± 2.3	(3.0-7.6)	5.4 ± 1.9	(3.6-7.3)	0.85
UA (mg/dL)	1.4 ± 0.9	(0.5-2.2)	1.3 ± 1.0	(0.4-2.3)	1.4 ± 0.8	(0.6-2.3)	0.71
Sugars, lipids, and pancreatic-associated enzymes							
GLU (mg/dL)	73.5 ± 20.9	(53-94)	66 ± 20.9	(45-87)	78.1 ± 19.9	(58-98)	0.10
TRIG (mg/dL)	93.3 ± 38.3	(55-132)	1,10.7 ± 38.1	(73-149)	65.5 ± 22.4	(43-88)	0.0003*
CHOL (mg/dL)	180.2 ± 40.9	(139-221)	1,73.7 ± 41.6	(132-215)	187.8 ± 35.4	(152-223)	0.30
AMY (U/L)	442.2 ± 124.3	(318-567)	444.7 ± 122.3	(325-569)	437.7 ± 141.0	(297-579)	0.88
Proteins							
TOT PROT (g/dL)	6.3 ± 0.4	(5.9-6.7)	6.3 ± 0.3	(6.0-6.6)	6.3 ± 0.4	(5.9-6.7)	0.69
ALB (g/dL)	4.1 ± 0.4	(3.7-4.4)	4.0 ± 0.4	(3.6-4.4)	4.2 ± 0.4	(3.9-4.6)	0.08
GLOB (g/dL)	2.2 ± 0.5	(1.8-2.7)	2.3 ± 0.4	(1.8-2.7)	2.3 ± 0.5	(1.8-2.8)	0.97
ALB:GLOB	1.8 ± 0.4	(1.4-2.3)	1.8 ± 0.4	(1.4-2.2)	1.9 ± 0.5	(1.4-2.3)	0.70
Electrolytes							
Na (mmol/L)	150.8 ± 6.6	(144-157)	151.7 ± 6.0	(146-158)	150.2 ± 7.6	(143-158)	0.53
Cl^- (mmol/L)	96.0 ± 6.6	(89-103)	96.1 ± 6.9	(89-103)	96.8 ± 6.2	(91-103)	0.74
K (mmol/L)	5.1 ± 0.8	(4.3-5.9)	5.3 ± 0.6	(4.7-5.8)	4.9 ± 1.0	(3.9-5.9)	0.24
PO_4 (mg/dL)	4.2 ± 0.9	(3.2-5.1)	4.1 ± 0.9	(3.2-4.9)	4.2 ± 1.1	(3.1-5.3)	0.73
Ca (mg/dL)	9.3 ± 1.2	(8.1-10)	9.3 ± 1.1	(8.2-10)	9.0 ± 1.2	(7.9-10)	0.54
Mg (mg/dL)	3.4 ± 1.0	(2.4-4.4)	3.7 ± 1.2	(2.5-5.0)	3.1 ± 1.0	(2.0-4.1)	0.57

may always be ideal to correlate these findings with acute-phase proteins and imaging aids (Harr et al., 2011; Harvey et al., 2018; Barreto et al., 2021). Studies by Harr et al. (2008) suggest that in manatees, the measurement of lactate dehydrogenase activity, specifically isoenzyme M4 (LDH 5), is recommended, allowing for a more selective indicator of muscle injury.

The total bilirubin ranges found in this study coincide with those reported in Amazonian, Antillean, and Florida manatees (Table 7). While this is the first study to consider indirect bilirubin levels and the ratio between total bilirubin and indirect bilirubin, the obtained values are similar to those reported in horses (Harr et al., 2008; Díaz et al., 2011; Mendonça et al., 2020). The

Table 7. Serum chemistry ranges for Antillean manatees from Colombia, Puerto Rico, Guyana, Mexico, Belize, and Brazil; Florida manatees; and Amazonian manatees (*Trichechus inunguis*). Colombia values included all samples (calves, subadults, and adults). Abbreviations for parameters are detailed in the "Methods" section. Significant differences using *p* values between Colombia and Puerto Rico, and Colombia and Brazil's Amazonian manatees are indicated with an asterisk (*). Columns with a silcrow (§) signify that the range values are minimum and maximum.

	Antillean manatees						Florida manatees		Amazonian manatees
Parameter	Colombia *n* = 45	Puerto Rico[a] *n* = 70	Guyana[b] *n* = 11	Mexico[c] *n* = 16	Belize[d] *n* = 82	Brazil[e§] *n* = 30	Florida[f] *n* = 27	Florida[g§] *n* = 55	Brazil[h, i] *n* = 24
Liver-associated enzymes and pigments									
LDH (U/L)	122-252	261-590*	--	--	--	--	94-372	--	111-278
DIR BILI (mg/dL)	0-0.3	--	--	--	--	--	--	--	--
IND BILI (mg/dL)	0.1-0.4	--	--	--	--	--	--	--	--
TOT BILI (mg/dL)	0.1-0.6	0.1-0.3*	0.2-0.4	0-0.4	-	0-0.1	0-0.2	0-0.1	0-0.9
Muscle-associated enzymes									
ALT (U/L)	13-40	8.3-23*	--	14-24	4.4-33	3.0-9.0	2.0-40	5.0-48	4.0-20
AST (U/L)	7.1-14	6.1-17	18-19	13-80	19-52	6.0-13	0-84	4.0-26	8.0-22
ALP (U/L)	75-177	58-99*	45-80	--	52-106	214-412	56-216	39-192	48-102
CPK (U/L)	33-206	68-132	75-228	78-191	--	--	18-729	51-2,966	72-242
Kidney-associated compounds and products									
BUN (mg/dL)	5.5-14	2.1-6.3*	1.6-6.4	3.1-13	1.7-9.5	13-21	0-21	7.2-77	28-54*
CREA (mg/dL)	1.3-2.3	1.1-1.9*	1.0-1.4	0-4.4	1.0-2.4	1.5-2.3	0.9-3.0	0.6-3.8	2.2-2.4*
BUN:CREA	3.0-7.4	1.4-2.8*	--	--	--	--	--	--	--
UA (mg/dL)	0.5-2.2	0.4-1.4*	--	--	--	1.6-2.2	--	--	0.8-1.4*
Sugars, lipids, and pancreatic-associated enzymes									
GLU (mg/dL)	53-94	73-110*	70-97	67-101	44-120	66-129	90-189	41-178	33-59
TRIG (mg/dL)	55-132	84-135*	--	82-134	50-131	--	--	5.4-40	82-188*
CHOL (mg/dL)	139-221	92-143*	--	134-210	88-170	148-243	77-396	38-132	134-284
AMY (U/L)	318-567	451-749*	--	--	--	--	1,493-3,900	--	1,190-1,636*
Proteins									
TOT PROT (g/dL)	5.9-6.7	6.4-7.5*	6.5-7.3	6.9-8.3	6.3-7.8	6.4-7.8	5.8-8.4	6.4-9.0	6.4-7.4*
ALB (g/dL)	3.7-4.4	3.5-4.4*	4.1-5.1	4.0-5.6	3.3-5.1	4.6-6.7	2.6-6.2	2.5-4.6	3.0-3.8*
GLOB (g/dL)	1.8-2.7	2.5-3.3*	--	2.3-3.3	--	--	1.5-3.3	3.3-5.4	4.2-5.0*
ALB:GLOB	1.4-2.3	1.1-1.7*	--	1.1-2.4	--	--	--	0.6-1.3	--
Electrolytes									
Na (mmol/L)	144-157	146-157	138-149	--	142-160	--	134-158	143-158	142-144
Cl^- (mmol/L)	89-103	93-106*	92-105	--	87-105	--	86-124	78-106	--
K (mmol/L)	4.3-5.9	4.6-5.9	4.2-5.0	--	4.6-6.0	--	4.1-6.5	3.8-6.3	3.9-4.4*
PO_4 (mg/dL)	3.2-5.1	4.8-7.0*	4.2-5.6	--	3.7-7.0	--	3.4-6.3	--	5.7-7.4*
Ca (mg/dL)	8.1-10	9.2-11*	9.6-11	--	9.1-11	--	8.2-11	--	11-15*
Mg (mg/dL)	2.4-4.4	--	--	--	3.9-6.5	--	--	--	5.7-7.9*
CO_2 (mmol/L)	--	17-36	13-18	--	-	--	25-43	4.0-41	--
AG (mmol/L)	--	17-39	30-37	--	--	--	--	15-59	--

[a]Mignucci-Giannoni & Alsina-Guerrero, 2022; [b]Converse et al., 1994; [c]Olivera-Gómez et al., 2012; [d]Sulzner et al., 2012; [e]Mendonça et al., 2020; [f]Bossart et al., 2001; [g]Harvey et al., 2007; [h]de Mello et al., 2011; [i]Maduro et al., 2020

importance of assessing total, indirect, and direct bilirubin is that clinicians can use these to rule out problems associated with hemolysis, liver disease, and bile duct disorders (Harr et al., 2008; Thrall et al., 2022). However, to establish this type of diagnosis, it is necessary to complement the bilirubin analytes with fecal occult blood tests, urinalysis, serum iron levels, coagulation profile, and protein panel tests (Ambrojó et al., 2013; Gelain & Bonsembiante, 2019; Harvey et al., 2019).

Muscle-Associated Enzymes—The muscle-associated enzyme panel showed that alanine aminotransferase has ranges similar to those described in other Caribbean manatees, Florida manatees, and even Amazonian manatees. However, these results are well above the levels reported for Antillean manatees in Brazil (Table 7). Nonetheless, these higher values are still normal considering that manatees may inhabit different aquatic habitats (Sulzner et al., 2012). Both alanine aminotransferase, aspartate aminotransferase, and creatinine phosphokinase are used as predictors of muscle problems in manatees but not as liver condition or disease indicators (Bossart et al., 2001; Sulzner et al., 2012; Mendonça et al., 2020). Instead, it has been suggested that sorbitol dehydrogenase, glutamate, and bilirubin levels provide a better diagnosis of liver abnormalities in manatees (Harr et al., 2008), although higher levels of alanine aminotransferase and aspartate aminotransferase can induce a process called cytolysis pattern, which, if prolonged over time, can cause liver problems and cell destruction. More histopathological and blood studies are required to corroborate this in species such as the manatee (Busto Bea & Herrero Quirós, 2015). In the Colombian manatee population, we found a significant difference in the alanine aminotransferase levels in adults and subadults, which might be related to greater physical activity in response to stress and reproductive phases (Mundim et al., 2007).

The aspartate aminotransferase levels in this study were close to those found in Antillean manatees from Puerto Rico and Brazil. However, ranges of aspartate aminotransferase in manatees from Guyana, Mexico, Belize, and Florida and in Amazonian manatees were higher than in Colombian manatees (Table 7). These high levels are directly related to growth in young and wild individuals, as has been previously documented in manatees, and are not associated with liver damage (Sulzner et al., 2012; Mendonça et al., 2020). However, high levels of this enzyme have been documented in manatees due to the use of serum dry chemistry analyzers, resulting in significantly higher and inaccurate aspartate aminotransferase and alanine aminotransferase levels (Harr et al., 2008). The use of wet chemistry in plasma has recently been suggested in association with optimized assays using coenzyme pyridoxal-5-phosphate (P5P: vitamin B_6) because it allows for a more precise measurement of total plasma aminotransferases, which would facilitate the diagnosis of muscle and liver diseases through its holoenzyme form (Harr et al., 2008). Under normal conditions, manatees have low plasma aspartate aminotransferase compared to other herbivore species. This could be due to processes of muscle catabolism (prerenal increase in creatinine) and rapid contraction in the muscles of manatees, which depend on anaerobic glycolysis and glycogenolysis for energy (Harvey et al., 2007). Such biochemical findings suggest decreased aspartate aminotransferase activity by metabolic pathways (Harr et al., 2008). According to Bossart et al. (2001), aspartate aminotransferase is used in the daily clinical diagnosis of manatees to evaluate muscle damage. However, increases in aspartate aminotransferase and alanine aminotransferase have not been observed in manatees with subacute or chronic muscle trauma, despite simultaneous increases in muscle-specific creatinine phosphokinase activity (Harr et al., 2006).

Alkaline phosphatase levels were similar to those found in manatees from Guyana, Belize, and Florida; however, these levels were high in the Antillean population of Puerto Rico and the Amazonian manatees from Brazil (Table 7). Such an increase has been related to physiological processes of bone growth in young manatees, which are always accompanied by this enzyme plus high phosphorus levels (Sulzner et al., 2012). Elevations in this enzyme have also been documented in adult manatees with diseases that result in increased osteoblastic activity (Harvey et al., 2007). The clinical utility of phosphatase in manatees as a hepatic indicator lacks diagnostic utility due to its wide range (Bossart et al., 2001). Presently, liver diseases have not been reported by clinical diagnosis in manatees.

Kidney-Associated Compounds and Products—The Colombian population of manatees presented blood urea nitrogen, creatinine, uric acid, and blood urea nitrogen:creatinine ratio values within the ranges described for the species in Caribbean countries and Florida. However, the blood urea nitrogen values differ from those found in Antillean manatees from countries such as Mexico and Guyana and from Amazonian manatees in Brazil, where it was found to be higher than that found in the Colombian population (Table 7). Under normal conditions, blood urea nitrogen values in manatees are lower than in other marine mammals, reflecting their herbivorous diet and the metabolism of urea in the lower part of the gastrointestinal tract rather than a pathological response (Bossart et al., 2001).

The high levels of blood urea nitrogen found may be due to a high protein intake in the diet such as the case of Amazonian manatees artificially nursed with milk (Sousa et al., 2016). However, it is essential to highlight that high levels of urea nitrogen in manatees above the reference values may be associated with prerenal azotemia secondary to diuresis and hemoconcentration, which can be induced by hypothermia, dehydration, and gastrointestinal bleeding (Martony et al., 2019). To be sure of the correct interpretation of this analyte, it is necessary to correlate the results with urinalysis, measurement of symmetrical dimethyl arginine (SDMA), coagulation, and occult blood tests (Pantoja et al., 2010; Davis & Walsh, 2018; Cabrias-Contreras et al., 2021).

Sugars, Lipids, and Pancreatic-Associated Enzymes—Glucose values presented were within the ranges found in manatees from Puerto Rico and Guyana. However, they differed from what was found in Antillean manatees from Mexico, Belize, and Brazil; Florida manatees; and Amazonian manatees, which presented higher values (Table 7). The low serum glucose values in the Colombian population compared to the rest of the Caribbean populations is considered a finding of no clinical importance because the individuals in this study were clinically healthy, with recent food consumption, and the samples were centrifuged and subsequently sent to the laboratory without the incidental consumption of glucose by white and red blood cells in an uncentrifuged sample (Bossart et al., 2001; Thrall et al., 2022). Clinically, low glucose levels are usually attributed to starvation, but in manatees, it is probably related to their low metabolism (Maduro et al., 2020), which is why they have less need for glucose to cover their metabolic needs. High serum glucose levels in manatees with the absence of other alterations may suggest stress processes, recent food intake, diabetes, and food availability by climatic seasonality (Ball, 2020; Maduro et al., 2020). However, to corroborate the increased or decreased presence of serum glucose, it is necessary to relate it to fructosamine values in the blood serum or plasma (Ball, 2020). We found that the serum amylase values presented in the Colombian population coincided with the ranges reported for Puerto Rico and Brazil in which low levels are associated with little physical activity and dietary variation (Mendonça et al., 2020). However, these results differ from those reported in the Amazonian species (Table 7). High levels of this enzyme are associated with variations in habitat and in calves and juvenile manatees (Bossart et al., 2001; Harvey et al., 2007; de Mello et al., 2011). When alterations of this enzyme are suspected, it will always be ideal to correlate the findings with the implementation of complete hematology and blood chemistry tests, urinalysis, coprology, and imaging aids (Harvey et al., 2007; Barreto et al., 2021; Cabrias-Contreras et al., 2021; Thrall et al., 2022).

Triglyceride levels in the Colombian population coincided with those reported for the Antillean subspecies. However, the triglyceride levels of the Amazonian species and the Floridian subspecies are higher than those found in Colombian manatees (Table 7). Low lipid levels have been reported in manatees without association with pathologies. Similarly, horses have recorded similar levels, particularly the pony breed (Carmo, 2009; Maduro et al., 2020; Witkowska-Piłaszewicz et al., 2021). High levels of triglycerides in serum and the absence of other blood disorders may suggest stress with physical exertion, which generates an increase in the lipid concentration due to insulin blockage and the hyperglycemic effect caused by circulating catecholamines and cortisol (Maduro et al., 2020). This leads to a negative energy balance, similar to what occurs in horses subjected to food fasting, with lipolysis and the mobilization of other energy sources (Dugat et al., 2010). We found significant differences between the triglyceride levels of subadult and adult animals. When comparing these results with ponies, it was possible to determine that such variations are present and increased in young animals such as the subadult manatees in this study. These levels decrease as the animals increase in age (Witkowska-Piłaszewicz et al., 2021).

Cholesterol levels were similar to those described for the subspecies in different Caribbean countries and for the Floridian subspecies. However, cholesterol levels in the Amazonian species were higher than those found in Colombian manatees (Table 7). Such findings could be related to reduced food intake during the dry season, which causes fat reserves to metabolize. The presence of high levels associated with the excessive consumption of aquatic plants rich in lipids during the dry season has also been documented (Colares et al., 2000; Maduro et al., 2020). The rainy weather season and excessive consumption of cholesterol-rich foods within rehabilitation programs may also contribute to cholesterol findings (Colares et al., 2000; Maduro et al., 2020). The use of plasma lipid profiles (acylcarnitines) and serum amyloid A as biomarkers of metabolic/liver and inflammation-specific problems has recently begun to be implemented (Griffin et al., 2021).

Proteins—The interpretation of protein panels provides essential information about the transport and elimination of nutrients, lipids, hormones, immunological aspects, vitamins, and minerals necessary for maintaining homeostasis in the organism (Willard & Tvedten, 2011). However,

their correct use must be correlated with specific laboratory panels and techniques such as metabolic profiles, acute phase proteins, and electrolyte panels (Ortíz et al., 2000; Harvey et al., 2018; Maduro et al., 2020). Proteomic profiling has recently begun to be implemented in manatees, which is associated with acute-phase inflammatory response, amyloid formation and accumulation, copper and iron homeostasis, the complement cascade pathway, and other critical cellular functions (Lazensky et al., 2020, 2021). Total proteins, albumin, globulins, and albumin:globulin ratio values are very close to the ranges reported in Florida and Antillean manatees. However, the values reported for the albumin:globulin ratio in this study differ from those found in Belize, which was higher than in Colombia. The latter is possibly associated with the presentation of dehydration, inflammation, or stress processes, which the wild individuals in the Belize study could be going through (Harvey et al., 2007, 2018; Silva et al., 2007, 2009; Mendonça et al., 2020; Thrall et al., 2022). It is currently considered that albumin/globulin levels are associated with serum amyloid A as a reliable marker of inflammation in manatees, as long as they are measured by serum or plasma electrophoresis, which will allow obtaining more precise serum concentrations (Harr et al., 2008, 2011; Cray et al., 2013).

Electrolytes—The ranges obtained in the population and the different categories of this study reveal that the electrolyte panel presents similar ranges to those reported in countries such as Puerto Rico, Belize, Mexico, Guyana, and Brazil, as well as in Florida. However, the calcium values obtained in Colombia were well below those reported for Amazonian manatees (Table 7). The high levels of calcium presented in the Amazonian species could be due to several causes, among them a high intake of freshwater aquatic plants rich in large amounts of calcium carbonate, mineral supplementation, climatic season, and little metabolic activation of vitamin D product in the dark waters common in the Amazon River and its tributaries. Another factor that directly influences the increase in calcium is the age category and sex of the manatees (Rosas et al., 1999). High levels of calcium have been documented in the calf or subadult manatees in the growth phase without pathological alteration (Millán-Sánchez, 1999; Colares et al., 2000; Siegal-Willott et al., 2013; Sousa et al., 2016; Ball, 2021). To corroborate these alterations, it is always essential to correlate these findings with thyroid profiles and protein panels by electrophoresis due to the direct relationship between albumin and urinalysis (Millán-Sánchez, 1999; Ortíz et al., 2000; Manire et al., 2003; Cabrias-Contreras et al., 2021). The magnesium levels in the study population and its different categories are below the values reported for the Amazonian species and Belize's Antillean and Floridian subspecies (Table 7). The low levels of magnesium found in Colombian manatees could be due to a deficiency of said mineral in the diet of these individuals. Such a finding has been reported in manatees both in captivity and in the wild and is often related to periods of lack of food availability due to climatic seasonality in the riverine or lacustrine complexes (Rosas et al., 1999). Since hypomagnesia is usually associated in other mammals with hipokalemia, decreased serum sodium, phosphate, and calcium, it is important to include these analytes as part of the chemistry panel to rule out diagnosing any possible ailment. The significant differences found between the Antillean manatee populations of Colombia and Puerto Rico, and the Amazonian manatees in Brazil are probably determined by factors such as age, type of feeding, size of the population, and environmental conditions (Silva et al., 2007; Sulzner et al., 2012; Mendonça et al., 2020). It is essential to mention that although the average values and deviations between these populations were compared in this study, the maximum and minimum ranges will allow for establishing a veterinary clinical diagnosis of an individual's health status (Brooks et al., 2022).

Conclusions

We determined the reference intervals of hematology and serum chemistry for manatees in Colombia and established these for different age and sex categories. No relevant clinical variations were found in hematological or biochemistry parameters due to the manatee's sex, and the only differences found did not present clinical relevance. Marked differences were found between age groups, mainly among younger animals with expected slightly faster metabolism than adult manatees. There were significant variations between hematological and blood chemistry values of the different country populations of West Indian manatees compared to Colombian manatees. Such variations are likely to be influenced by evolutionary history as well as environmental factors such as saline and estuarine vs riverine or lacustrine environments. Antillean manatees from Colombia live primarily in five major rivers (Atrato, Magdalena, Meta, San Jorge, and Sinú), their tributaries, and associated wetlands in this South American country (Millán-Sánchez, 1999; Caicedo-Herrera et al., 2004; Caballero et al., 2021; Debrot et al., 2022), while other Antillean manatee populations, particularly in Belize, Brazil, Mexico, and Puerto Rico, live

primarily in marine or brackish estuarine waters (Morales-Vela et al., 2024). Profound differences were observed between the marine manatees of Puerto Rico and the riverine and lacustrine manatees of Colombia. However, marked differences were also evident between the sister species *T. manatus* from Colombia and *T. inunguis* from Brazil, both living in river environments. While marine and lotic ecosystems present obviously different physiological challenges for manatees, there are marked water physicochemical differences between the rivers of Colombia and the Amazon River and its tributaries in Brazil (Mojica-Figueroa et al., 2014). Thus, differences between marine and freshwater manatees in terms of their hematology and blood chemistry are accounted for, as well as variations between different river environments such as the rivers of the Andean and Caribbean valleys in Colombia in comparison with the Amazon River. The latter reinforces the need for population-specific reference intervals for aquatic species such as the manatee.

The population-specific reference intervals obtained in this study will enable us to periodically evaluate the health status of an individual, as well as to be able to detect early diseases in manatees that enter rescue programs or are kept under human care, which would lead to the best establishment of appropriate therapeutics when warranted. Additionally, implementing routine blood tests could reduce costs associated with the treatment of advanced diseases by early and correct diagnosis, and eliminate possible recurrences of diseases due to inadequate diagnosis and treatments without timely manner and correct diagnostic tests such as hematology and blood chemistry.

It is recommended that future studies correlate these blood tests with specific panels like acute phase proteins to detect problems at the muscle level and inflammation; measurement of the analyte dimercaptosuccinic acid (DMSA) as a predictor of kidney problems; serum amyloid A, acute phase proteins, and proteomic profiles as a biomarker of chronic trauma infection; a D-Dimer test to measure coagulation; and sorbitol dehydrogenase, glutamate, iron, mineral concentration, and coagulation tests. We further recommend conducting wild manatee health assessments as this information will yield important species management schemes needed due to the multiple anthropogenic and environmental threats that manatees face today, including from invasive species such as hippopotamuses (*Hippopotamus amphibius*; Castelblanco-Martínez et al., 2021; Subalusky et al., 2021), which put the Colombian manatee's health and ultimate survival at risk.

Acknowledgments

Manatee rescue, rehabilitation, and specimen collection were conducted under the authority of the Instituto Nacional de los Recursos Naturales Renovables y del Medio Ambiente (INDERENA; between 1992 and 1993), the Ministerio del Ambiente (between 1994 and 2021), and the Corporación Autónoma Regional de los Valles del Sinú y San Jorge (CVS), Corporación Autónoma Regional del Canal del Dique (CARDIQUE), Corporación Autónoma Regional del Sur de Bolivar (CSB), Corporación Autónoma Regional del Atlántico (CRA), Corporación Autónoma Regional de Sucre (CARSUCRE), and Corporación Autónoma Regional del Magdalena (CORPOMAG) issued to Red Caribeña de Varamientos or Fundación Omacha. The initial part of this study was partially funded by the Save the Manatee Club and Red Caribeña de Varamientos. We gratefully acknowledge the assistance of the Club Amigos del Manatí, the Botero-Maya family, and Fundación Omacha's technicians, students, fishers, and volunteers in the rescue and rehabilitation of the manatees from which blood samples were taken for this study. We also appreciate the excellent work of Laboratorio Clínico y Patología Bernardo Espinosa and Laboratorio Clínico Veterinario BioVet for their continued support and analysis of blood samples. We thank I. Méndez and R. Ortíz for their assistance and review of the manuscript.

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