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SHORT COMMUNICATION

Development of reference intervals for serum biochemistry and haematology of juvenile green sea turtles (*Chelonia mydas*) in a Thai rehabilitation centre

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No reference intervals for serum biochemistry and haematology of sea turtles in Thailand exists to assist veterinarians who are responsible for sea turtle health management and treatment. This study determined serum biochemistry and basic haematology of healthy juvenile green sea turtles (n = 92) in captivity in Thailand following the American Society for Veterinary Clinical Pathology (ASVCP), Quality Assurance and Laboratory Standards Committee (QALS) guidelines for the determination of reference intervals in veterinary species. Biochemistry tests, including blood urea nitrogen, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were analysed using an IDEXX VetTest Chemistry Analyzer. Haematology parameters were measured manually using a microhaematocrit for packed cell volume (PCV), Neubauer counting chamber for red blood cell count and cyanmethemoglobin method for haemoglobin concentration. mean corpuscular volume and mean corpuscular haemoglobin concentration were calculated using the PCV, red blood cell count and haemoglobin. Turtles in this study were found to have higher mean values for PCV (28.70%), haemoglobin (92.13 g/L), mean corpuscular haemoglobin concentration (327.03 g/L), uric acid (247.15 μmol/L), alanine aminotransferase (16.53 IU/L), aspartate aminotransferase (209.44 IU/L), and alkaline phosphatase (245.08 IU/L) compared to sea turtles in Brazil. The reference intervals established using high numbers of healthy turtles in this study will assist veterinarians with diagnostic and treatment decisions when evaluating laboratory results for juvenile green sea turtles.

Keywords green sea turtles; haematology; juvenile; rehabilitation center; serum biochemistry

Abbreviations ASVCP, American Society for Veterinary Clinical Pathology; Hgb, haemoglobin; IUCN, International Union for Conservation of Nature and Natural Resources; PCV, packed cell volume; QALS, Quality Assurance and Laboratory Standards Committee; RBC, red blood cell

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The green sea turtle (*Chelonia mydas*) is included on the Red List of the International Union for Conservation of Nature and Natural Resources (IUCN).[1] Some causes of the decline of green sea turtle numbers are environmental change, marine waste caused by human activities, hunting for consumption by humans and other predators and habitat destruction by fishing activities and water pollution.[2]

Worldwide conservationists are working to conserve green sea turtles. Physical examination and blood analysis assist in health evaluation of captive turtles.[3] Studies on biochemistry and haematology of sea turtles are limited; however, some studies on the serum biochemistry and haematology in adult and juvenile sea turtles including turtles affected by entanglement, trauma and clinical health issues have been reported.[4,5] These reveal variable serum biochemistry reference intervals for different species and geographic locations in part reflecting variable health status at the time of blood collection. This study aims to determine serum biochemistry and basic haematology of healthy juvenile green sea turtles in captivity in Thailand following the American Society for Veterinary Clinical Pathology (ASVCP), Quality Assurance and Laboratory Standards Committee (QALS) guidelines for the determination of reference intervals in veterinary species.[6] These reference intervals will be valuable to identify and diagnose disease in clinically unwell turtles.

Ninety-two healthy juvenile green sea turtles (*C. mydas*) aged from 9 months to 1 year housed at the breeding and rehabilitation center, Marine and Coastal Resources Research and Development Institute, Rayong province, Thailand, were included in this study. A government veterinary officer collected blood as part of a routine health check. The health check included a physical examination, measurement of standard carapace length, carapace width, and weighing each sea turtle. Four milliliters of blood were collected from the dorsal cervical sinus using a 22 gauge needle and 10 ml syringe and placed into plain tubes and EDTA tubes (two milliliters each tube). After blood clotting in the plain tube, serum was separated by centrifugation at 12,000 *g* for 120 s. IDEXX VetTest Chemistry Analyzer, USA, was used for serum biochemistry evaluating creatinine, uric acid, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Blood samples in the EDTA tubes were tested by manual haematological methods, following Salakij's protocol.[7] Packed cell volume (PCV) was measured after the blood was transferred to microcapillary tube and centrifuged at 10,000 *g* for 5 min. Red blood cell (RBC) concentration was

Table 1. **Morphometric and reference intervals for haematology and biochemistry from juvenile green sea turtles (*Chelonia mydas*) in Thailand**

Analyte	n	Lower limit	Lower limit 90% CI	Upper limit	Upper limit 90% CI	Interval method
SCL (cm)	92	17.77	17.50–18.16	23.84	23.00–24.50	NP
CW (cm)	92	16.16	15.00–17.00	21.97	21.00–22.00	NP
Weight (kg)	92	0.71	0.64–0.77	1.61	1.54–1.67	Parametric
PCV (%)	91	16.60	14.00–20.00	34.00	34.00–34.00	NP
MCV (fL)	92	293.01	268.89–320.16	628.43	601.36–654.19	Parametric
Hgb (g/L)	55	59.21	51.35–66.84	120.68	111.42–129.28	Robust
MCHC (g/L)	62	85.07	28.03–142.69	496.50	436.65–560.09	Robust
MCH (pg/cell)	60	84.55	82.86–90.04	231.51	213.46–232.01	NP
Creatinine (μmol/L)	89	44.20	42.00–47.68	177.24	163.32–208.62	NP
Uric acid (μmol/L)	59	99.13	71.34–127.27	395.18	367.82–423.14	Parametric
BUN (mmol/L)	92	1.56	1.03–2.08	8.42	7.90–8.92	Parametric
ALT (IU/L)	86	5.64	5.53–6.70	45.33	32.26–51.40	NP[a]
AST (IU/L)	85	118.02	97.57–139.42	315.92	297.85–334.29	Robust
ALP (IU/l)	92	94.20	72.84–116.67	395.95	373.90–417.79	Parametric

[a] Distribution for ALT (IU/L) was shown to be symmetrical. However, a nonparametric method was applied due to interval values being outside the range of possible values (i.e. negative) when a robust interval method was applied.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; CW, carapace width; Hgb, Haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NP, nonparametric; PCV, packed cell volume; SCL, standard carapace length.

determined manually with a Neubauer counting chamber after the blood was diluted 200 times with Natt and Herrick solution. Haemoglobin (Hgb) concentration was determined by the cyanmethemoglobin method, after free RBC nuclei were removed by centrifugation. The mean corpuscular volume, mean corpuscular haemoglobin concentration, and mean corpuscular haemoglobin were calculated from the PCV, RBC count, and Hgb concentration.[8]

Table 2. **Comparison of haematology and serum biochemistry of juvenile green sea turtles (*Chelonia mydas*) in Thailand and Brazil including descriptive statistics of blood parameters from juvenile green sea turtles**

	This research results from juvenile Green sea turtles in Thailand					Results from juvenile Green sea turtles in Coroa Vermelha (Brazil), Miguel et al., 2022				
Blood parameters	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
SCL (cm)	92	21.01	1.46	17.50	24.50	69	45.30	7.10	30.00	59.30
CW (cm)	92	19.02	1.38	15.00	22.00	69	40.80	6.10	28.00	53.70
Weight (kg)	92	1.16	0.22	0.51	1.63	69	10.00	4.79	2.70	21.70
PCV (%)	91	28.70	4.60	14.00	34.00	69	20.00	6.00	6.00	40.00
MCV (fL)	92	460.72	83.98	290.91	684.21	69	623.00	180.00	242.00	1042.00
Hgb (g/L)[a]	55	92.13	15.09	68.96	149.61	69	60.50	19.80	20.00	108.00
MCHC (g/L)[a]	62	327.03	97.48	231.86	723.98	69	320.00	99.30	141.00	750.00
MCH (pg/cell)	60	142.99	35.71	82.86	232.01	69	193.00	62.00	53.00	444.00
Creatinine (μmol/L)[a]	89	95.87	36.00	44.20	208.62	69	NA	NA	NA	NA
Uric acid (μmol/L)[a]	59	247.15	73.33	139.18	477.03	69	54.72	31.52	2.38	142.75
BUN (mmol/L)[a]	92	4.99	1.72	1.75	9.07	69	9.07	15.49	1.89	89.25
ALT (IU/L)	86	16.53	8.36	5.53	51.40	69	3.35	1.49	1.00	9.60
AST (IU/L)	85	209.44	47.61	51.60	294.00	69	112.00	61.00	30.90	428.00
ALP (IU/L)	92	245.08	75.55	72.20	439.00	69	16.10	8.36	1.40	39.00

[a] Hb, MCHC, creatinine, uric acid and BUN results have been converted from U.S. unit into SI unit.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CW, carapace width; Hgb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NA, non-applicable result; PCV, packed cell volume; SCL, standard carapace length.

Calculation of reference intervals was performed using Reference Value Advisor v2.1.[9] The analytical procedure followed the steps outlined by Friedrichs' team.[6] Visual inspection of histograms suggested that data for some analytes were non-normally distributed. As outlier tests are more accurate when data approximate a Gaussian distribution, outlier tests were performed on Box-Cox transformed values. Two outlier tests were performed: the Dixon-Reed test and Tukey's test. The Dixon-Reed test identifies if the lower or upper most extreme value in a distribution is an outlier. Tukey's test designates outliers as either mild or extreme. Identified outlying values (both mild and extreme) were removed from the dataset. After removing outlying values, outlier tests were performed again. No outliers were detected on Box-Cox transformed values during this second round of outlier screening. After removal of outliers, normality was tested via the Anderson-Darling test and symmetry was tested via McWilliams' method.[10] The normality and symmetry assumptions were considered to be violated when $P < 0.05$.

Following the QALS recommendations for determining reference intervals with sample sizes of between 40 and 120, parametric methods were applied when analytes were normally distributed; robust methods were used when data were non-normal but symmetrical; and nonparametric methods were used when data were neither normally distributed nor symmetrical.[6] In all cases, 90% confidence intervals around reference values were created via a nonparametric bootstrap method.[9]

Reference intervals with 90% confidence intervals established in this study and descriptive statistics are presented in Table 1. The haematology and serum biochemistry results from this study were compared to the results from sea turtles in Brazil[3] (Table 2) based on the similarity of sea turtle species. Higher mean values of PCV (28.70%), Hgb (92.13 g/L), mean corpuscular haemoglobin concentration (327.03 g/L), uric acid (247.15 μmol/L), alanine aminotransferase (16.53 IU/L), aspartate aminotransferase (209.44 IU/L), and alkaline phosphatase (245.08 IU/L) were identified in Thai green sea turtles compared to juvenile green sea turtles in Brazil. These differences may be related to differences in environmental conditions, diet, and age.

Haematology and biochemistry parameters are a valuable tool to monitor the health status of turtles and establish the cause of disease in conjunction with clinical signs when they are unwell. This is, however, only possible once accurate reference intervals reflecting physiological and ecological conditions are established.

This study determined reference intervals of serum biochemistry and haematology data in juvenile green sea turtles in captivity in Thailand which may assist veterinarians to evaluate the blood results from stranded sea turtles, monitor sea turtle health, and captive management. Accurate reference intervals established using a significant number of sea turtles helps veterinarians to avoid false negative or false positive diagnosis when evaluating results from sick animals. This study can assist veterinarians to make appropriate diagnostic and treatment decisions when evaluating laboratory results for sea turtles in the future.

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