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Hematological reference values for *Alouatta palliata* (Primates: Atelidae) and *Cebus imitator* (Primates: Cebidae) from the Pacific Coast of Costa Rica

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ABSTRACT

Introduction: Wildlife hematological patterns are fundamental for health monitoring, and allows elucidating variations both within and between populations. Among these, hematological parameters are particularly valuable to evaluate the health status of neotropical primate species in the wild.

Objective: To define hematological reference values for two species of monkeys in Costa Rica.

Methods: During 2014, we collected blood samples from free-ranging mantled howler monkeys, *Alouatta palliata* (17 females, 18 males) and white-faced capuchin monkeys, *Cebus imitator* (5 females, 7 males) in seven localities of the Costa Rican Pacific coast.

Results: For both species, the hematological values were higher in males, and howler monkey populations differed significantly except for platelets.

Conclusions: These hematological values, which differ by sex and locality, will help evaluate the health status of these neotropical primate populations.

Key words: Neotropical primates; mantled howler monkey; white-faced capuchin monkey; hematology; Costa Rica.

Neotropical primates (NP; suborder Platyrrhini) have a wide geographical distribution, that includes the Southern part of Mexico, Central and South America, and are represented by a huge variety of species, with a remarkable

diversification in their biology, life history and behavior (Rosenberger & Hartwig, 2001). In Costa Rica, four different species of NP are distributed, including mantled howler monkey (*Alouatta palliata*), white-faced capuchin



monkey (*Cebus imitator*), spider monkey (*Ateles geoffroyi*) and squirrel monkey (*Saimiri oerstedii*). Three of these are at risk of extinction (mantled howler, spider and squirrel monkeys), and one (white-faced capuchin monkey) is a threatened or vulnerable species, according to the Costa Rican legislation (Decree No. 2635) and the International Union for Conservation of Nature (IUCN) (Cuarón et al., 2008; MINAE, 1997; Wong et al., 2008). At the same time, these three species are included in appendix 1 of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES, 2021) and the white-faced capuchin monkey has yet to be classified.

Costa Rica is considered one of the countries with the greatest diversity in the world and has a wide variety of microclimates and life zones that favor its notable levels of biodiversity. However, the biological diversity of the country is currently threatened by the illegal extraction of wood, the conversion of forests for agricultural activities or by tourist and real estate development, which strongly affect the connectivity between forest patches (Calvo-Obando & Ortis-Malavassi, 2012). As natural environments are transformed, through anthropogenic activities, the natural habitat of the NP is either reduced in size or fragmented, directly affecting them. Furthermore, as monkey populations concentrate, troops decrease in number of individuals, causing a reduction of the genetic pool, an increase in stress, promoting immunosuppression and the eventual transmission of diseases (Gilbert & Dodds, 1987).

Given this, counting on reference data about health status, genetic variety, biological characteristics, among other variables, are a priority in conservation programs. Hematological reference values are a valuable tool to help determine the health status of wild populations (Takeshita et al., 2011). This data may be used as comparison patterns between populations that are distributed in landscapes of different forest types or captive environments. And, in addition, may also be useful for reintroduction and management programs (Sánchez-Sarmiento et al., 2014).

Health studies and the establishment of normal blood values research have increased over the last decades, providing an important amount of information on normal parameters for several species and are valuable tools for assessing and monitoring the health and resilience of wild populations (Maceda-Veiga et al., 2015). This information helps identify potential effects of diseases, pollutants, or other changing environmental conditions that would be difficult to understand without knowledge of normal species variations in blood variables. These kinds of studies usually include variables that are used in veterinary medicine and are assumed to have the same utility in wild populations (Arguedas et al., 2021).

Health status evaluations, taking in consideration hematological parameters, have been done for several species of NP and are used as a reference for the comparisons between or within species (García-Feria et al., 2017). Nevertheless, there are few studies published on the hematology of NP in natural environments from Central America, due to lack of project financing or even research interest. In this study, we evaluate some hematological analytes of mantled howler monkeys (*Alouatta palliata*) and white-faced capuchin monkeys (*Cebus imitator*) in the wild coming from natural environments from Costa Rica, by analyzing such parameters and comparing the results obtained between genders and the populations sampled.

MATERIALS AND METHODS

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page. All procedures for trapping and handling NP were approved by bioethics and welfare permits given by the University of Costa Rica and the capture permits given by SINAC (Resolution No. 27 2013 SINAC).

Animal Sampling: During 2014, mantled howler and white-faced capuchin monkeys from Costa Rica were captured (Fig. 1). Capture was done using 3.3-1.1 mg/kg Zoletil 50® (Virbac) or Ketamine 10 % (Bremer

Pharma GmbH, 5-20 mg/kg) with Xylazine 2 % (Virbac, 0.5-2.0 mg/kg) (Glander et al., 1991; Varela, 2006; West et al., 2007) in darts (PneuDart. Inc, Type P, 1cc) with a compressed gas rifle (PneuDart. Inc, model X-Caliber Gauged CO₂). Lateral faces of triceps and femoral quadriceps were the target of injection (Glander et al., 1991). Vital signs, including heart rate, respiratory rate, body temperature, oximetry, and mucous membrane color were observed during immobilization. A physical examination was performed on the animals looking for abnormalities that could indicate illness. When animals were fully recovered from anesthesia, they were released. General body condition of the individual was evaluated using a five-point scale.

Laboratory Analysis: Once the individual was anesthetized, 2-4 ml blood samples were extracted from the femoral vein using the Vacutainer® system. Samples were collected in EDTA tubes and transported to the laboratory at 4 °C. At the lab, the following hematological parameters were evaluated using Sysmex KX 21 equipment, (Sysmex Corporation, Japan): red blood cell count (RBC, 10⁶/mm³), hemoglobin (Hb, g/dl), hematocrit (Hct, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCCH, g/l), platelet count (PLT, 10³/mm³), and white blood cell count (WBC, 10³/mm³).

Statistical analysis: For each parameter measured, Mann-Whitney test was performed, depending on the normality of data a Wilcoxon and t student tests were performed in order to determine if there were significant differences between females and males from each species sampled. Also, for mantled howler monkeys, an ANOVA tests was performed to determine if there are significant differences between sampled populations. Statistical analysis was done in R program (R Core Team, 2016) with a < 0.05 significance level.

RESULTS

A total of 35 (17 females and 18 males) mantled howler monkeys and 12 (5 females and 7 males) white-faced capuchin monkeys were captured in seven localities from Costa Rica, five localities for mantled howler monkeys, including: San Lucas island (9°56'24.28" N & 84°54'15.24" W), Nicoya (10°08'40" N & 85°27'22" W), Hermosa beach (4°46'00" N & 85°16'00" W), Manuel Antonio (9°22'32" N & 84°08'09" W), Curú (9°47'35.55" N & 84°55'32.49" W), and 2 localities for white-faced capuchin monkeys, including Manuel Antonio (9°22'32" N & 84°08'09" W) and Curú (9°47'35.55" N & 84°55'32.49" W) (Fig. 1), (Table 1). All individuals appeared to be healthy and did not present any sign of disease.

For mantled howler monkeys, the measures of Hemoglobin (Hb), Hematocrit (Htc), red blood cells count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Platelets (PLT) and White blood cell count (WBC) were higher in males than females. For white-faced capuchin monkeys, values of Hb, Htc, RBC, PLT and WBC were also higher for males than females, but MCV, and MCH values were similar for both. Nevertheless, no significant differences were found between males and females for any of the parameters analyzed in both species (Table 2).

Significant differences for mantled howler monkeys and in all the parameters evaluated

TABLE 1
Distribution of individual mantled howler monkeys (*Alouatta palliata*) and white-faced capuchin monkeys (*Cebus imitator*) according to their original location

Capture site	Species	
	Mantled howler monkey (<i>Alouatta palliata</i>)	white-faced capuchin monkey (<i>Cebus imitator</i>)
Curú	1	8
Manuel Antonio	2	4
Nicoya	9	0
Hermosa Beach	4	0
San Lucas Island	19	0
Total	35	12

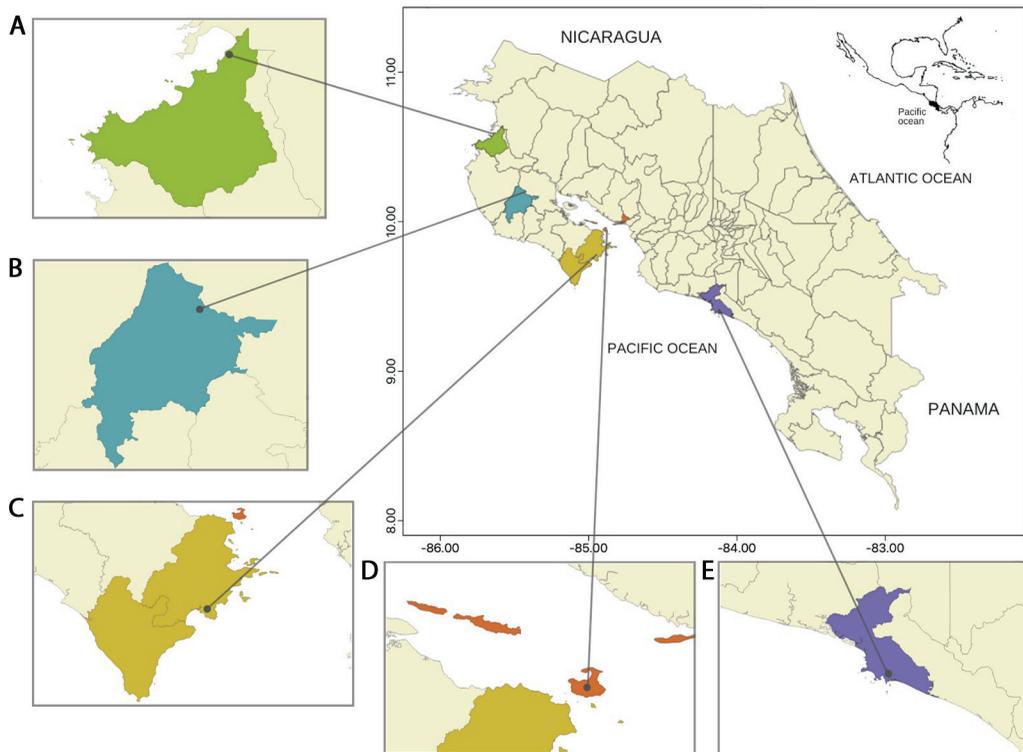


Fig. 1. Location of field sites in Costa Rica: **A.** Hermosa Beach. **B.** Nicoya. **C.** Curú. **D.** San Lucas Island. **E.** Manuel Antonio.

were determined, except for PLT. Individuals from the Nicoya locality were the individuals that presented the highest scored hematological values. At Curú just one individual was captured, so it was not included in the analysis (Table 3).

For the other hematological parameters evaluated, 3/35 (8.6 %) of mantled howler monkeys and 3/12 (25 %) of white-faced capuchin monkeys presented WBC counts above 10 400/mm³. In the same way, 16/35 (45.7 %) of mantled howler monkeys and 2/12 (16.7 %) of white-faced capuchin monkeys presented PLT values above 250 000/mm³, and one howler monkey presented an abnormally low value of platelets (17 300/mm³).

DISCUSSION

Actual information related to the biology, ecology, health and diseases supports and

facilitates programs related to the management, medicine and conservation of wildlife populations (Varela, 2006). Changes in landscape, habitat fragmentation and the anthropogenic activities directly affect the wellness of NP, and this may be reflected in the hematological parameters. In order to obtain and compare the reference patterns of hematological parameters for mantled howler and white-faced capuchin monkeys from Costa Rica, free-living individuals of both species were captured at different geographical regions of Costa Rica.

The results of this study are the first hematological parameters reported for mantled howler monkeys (*A. palliata*) in Costa Rica. When these results are compared with values published for free living *A. palliata* individuals from Tabasco, Mexico (Canales-Espinosa et al., 2015), and for *A. pigra* individuals from Balancán, México (García-Feria et al., 2017), differences in the averages of parameters

TABLE 2
Hematological values of mantled howler monkey (*Alouatta palliata*) and white-faced capuchin monkey (*Cebus imitator*) in Costa Rica

Parameters	Mantled howler monkey					White-faced capuchin monkey						
	Females		Males			Females		Males				
	X ± SD	Median	MIN-MAX	X ± SD	Median	MIN-MAX	X ± SD	Median	MIN-MAX	X ± SD	Median	MIN-MAX
Hb (g/dl)	11.1 ± 1.4	10.9	9.0-13.8	11.9 ± 1.1	11.9	9.2-14.0	11.7 ± 1.6	11.4	10.2-14.0	12.2 ± 1.7	12.0	10.6-15.3
Hct (%)	37.9 ± 5.7	37.7	30.5-47.5	41.2 ± 4.6	41.0	30.8-48.4	40.5 ± 5.8	38.6	34.6-50.9	42.6 ± 6.2	43.5	36.0-52.8
RBC (x 10 ⁶ /mm ³)	4.0 ± 0.4	4.0	3.3-4.8	4.1 ± 0.5	4.2	3.1-5.0	5.2 ± 0.6	5.4	4.2-5.9	5.5 ± 0.7	5.2	4.6-6.7
MCV (fl)	95.5 ± 6.5	96.6	78.2-106.6	98.8 ± 4.5	98.1	91.3-109.0	78.7 ± 6.8	79.0	71.0-89.8	78.7 ± 7.7	82.9	66.9-86.3
MCHC (g/dl)	29.5 ± 1.5	29.1	27.2-32.5	29.0 ± 1.4	28.9	25.2-31.6	28.9 ± 0.8	29.2	27.5-29.8	28.9 ± 0.9	29.0	27.6-30.0
MCH (pg)	28.0 ± 1.8	28	22.1-30.4	28.6 ± 1.7	28.5	23.5-31.8	22.7 ± 1.5	22.3	21.0-24.7	22.6 ± 2.0	23.3	19.9-24.4
PLT (x 10 ³ /mm ³)	215.4 ± 72.5	209	86-333.0	220.7 ± 71.6	238	17.3-329.0	150.0 ± 85.4	128	49.0-280	219.6 ± 132.8	214	44.0-325.0
WBC (x 10 ³ /mm ³)	8.1 ± 2.4	7.8	2.9-12.7	8.7 ± 3.4	8.1	4.6-19.9	8.9 ± 4.2	9.6	3.5-13.8	11.1 ± 4.7	10.2	5.1-18.2

Hemoglobin (Hb), Hematocrit (HCT), red blood cells count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), Platelets (PLT) and White blood cell count (WBC).

measured are evident. Nevertheless, when our results are compared with the ones reported for Mexican howler monkeys, values obtained are within the maximal and minimal ranges described (Canales-Espinosa et al., 2015).

For white-faced capuchin monkeys, the hematological parameters obtained in this work are similar to those reported by Bernal-Valle et al (2020), where 16 out of 25 individuals also from Costa Rica. The median value for HCT reported by Bernal-Valle et al (2020) is 40.5 % for males and 37.5 % for females, compared with the values of 43.5 and 38.6 % obtained in this work. In the same way, the hemoglobin values obtained for males and females in both studies are similar (12.7 g/dl and 10.5 g/dl compared with 12 g/dl and 11.4 g/dl respectively). An important difference between both studies lies in the values obtained for white blood cells, where Bernal-Valle (2020) reports median concentrations of 5 700 WBC/ mm³ in females, compared with the values of 8 900 WBC/mm³ obtained in the present study. It is important to highlight those average values obtained in this study for white-faced capuchin monkeys are similar to the ones reported by Larsson et al (1999) in Brazil. Nonetheless, since different hematological analyzers were used in all the studies mentioned, it is important to always take this bias in account when comparing hematological values.

There are no significant differences between genders for any of the parameters evaluated in both species of monkeys studied. However, this data contrasts with those reported by other researchers, where values observed for males are greater than those reported for females in most of the parameters analyzed (Flaiban et al., 2009; García-Feria et al., 2017; Kagira et al., 2007; Larsson et al., 1999; Moore, 2000; Sánchez-Sarmiento et al., 2014; Takeshita et al., 2011; Wirz et al., 2008). This might be due, principally, to hormonal influence, since testosterone stimulates erythropoiesis, while estrogen inhibits it (Kagira et al., 2007; Wirz et al., 2008) or to effects of the menstrual cycle, pregnancy period and breastfeeding.



TABLE 3
Hematological values of mantled howler monkey (*Alouatta palliata*) according to the sampling site in Costa Rica

Parameters	Curú	San Lucas Island	Manuel Antonio	Hermosa Beach	Nicoya
	1 individual	X ± SD	X ± SD	X ± SD	X ± SD
Hb (g/dl)*	9	11.6 ± 1.3	11.6 ± 0.2	9.8 ± 0.2	12.2 ± 0.9
Hct (%)*	31.9	39.8 ± 4.8	37.9 ± 1	31.3 ± 0.3	43.8 ± 3.4
RBC (x 10 ⁶ /mm ³)*	4.1	4.1 ± 0.5	4.0 ± 0.2	3.5 ± 0.1	4.3 ± 0.4
VCM (fl)*	78.2	98.1 ± 2.4	95.3 ± 3.3	90.1 ± 1.7	101.4 ± 5.3
MCHC (g/dl)*	28.2	29.3 ± 1	30.5 ± 0.2	31.4 ± 0.3	27.8 ± 1.1
HCM (pg)*	22.1	28.7 ± 1.1	29 ± 1.2	28.2 ± 0.4	27.9 ± 2.2
PLT (x 10 ³ /mm ³)	86	239.5 ± 57.9	146.5 ± 23.3	190.5 ± 70.7	217.5 ± 86.6
WBC (x 10 ³ /mm ³)*	2.9	8.8 ± 3.1	12.8 ± 4.2	7.4 ± 0.4	8.4 ± 2.7

Hemoglobin (Hb), Hematocrit (HCT), red blood cells count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), Platelets (PLT) and White blood cell count (WBC), (*) ANOVA $P < 0.05$.

Differences between population may have occurred because of the excitement and tension that wild animals suffer during its capture, the individual may show signs of acute stress, known as “alarm reaction,” which may be the cause of an increase in the values of various parameters, including RBC, Hb, HCT, MCV, MCH, PLT and WBC (Thrall, 2004). In normal conditions, stress is an adaptive stimulus for a changing environment (Fowler, 1978) and causes beneficial metabolic changes for the protection against external aggressions (Cubas, 2007). On the other hand, other factors may cause differences in some hematological parameters, for example, some degree of dehydration can be a reason to have higher HCT, Hb and RBC, which can be suspected from animal coming from drier regions such as dry forest environments, like Nicoya.

Some of the individuals that had WBC higher than 10 000/mm³, can be because of an inflammatory process, bacterial infection or a severe stress reaction during capture (Thrall, 2004), further studies evaluating leukocyte response in these species have to be done in order to confirm these changes.

Apparent thrombocytosis (>250 000/mm³) here found in 16 howler monkeys and 2 capuchin monkeys, one cause of this, is reactive thrombocytosis, which is driven by elevated

endogenous levels of thrombopoietin, interleukin-6, other cytokines, or catecholamines that may be produced in inflammatory, infectious, or neoplastic conditions or in situations of stress (Schafer, 2004). Another finding was that one individual of howler monkey, presented an abnormally low platelet count, this may be, it can be due several reasons, for example, infectious agents or immune-mediated disorders (Thrall, 2004), or incidental, like platelet clumping due to venipuncture, which can be this case, since this particular individual, showed values within the normal ranges in the sample.

The difference in the hematological parameters evaluated might also be associated with other factors, including the type of feeding, diet and environmental factors among others. Health evaluations of free-ranging primates provides important information about the incidence and prevalence of infectious diseases, as well as their general health status and allows elucidating variations both within and between populations (Riviello & Wirz, 2001; Takeshita et al., 2011).

Health evaluations should not be limited to epidemiological surveillance and should also consider animals' clinical and physiological parameters (Sánchez-Sarmiento et al., 2014). For example, normal hematological values are

of special value in Costa Rican primates, since they frequently suffer from car accidents and electrocutions, Several species of Costa Rican wildlife are suffering terrible injuries along the power lines and transformers in rural areas of the country., and hematological parameters can be a way to predict (up to a point) the possible outcome of the patients and to be able to treat the animal in a more comprehensive way (Sánchez-Murillo & Arguedas, 2021). Besides, nonhuman primates exhibit a pronounced stress when they are confined (Suleman et al., 2000), and require a long period of time for adapting to captivity. Thus, hematological parameters may take even months to stabilize (Kagira et al., 2007).

Hematological parameters allow researchers and wildlife managers to predict and understand different disease processes, stress levels and hydration status of wild populations and thus, determine whether a population faces any stress related to environmental changes or anthropogenic causes. At the same time, these results can be used as a baseline when NP must be managed in captivity or during rehabilitation.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

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RESUMEN

Valores de referencia hematológicos para *Alouatta palliata* (Primates: Atelidae) y *Cebus imitator* (Primates: Cebidae) de la costa Pacífica de Costa Rica

Introducción: Los patrones hematológicos de la vida silvestre son fundamentales para el monitoreo de la salud y permiten dilucidar las variaciones tanto dentro como entre poblaciones. Entre estos, los parámetros hematológicos son particularmente valiosos para evaluar el estado de salud de las especies de primates neotropicales en la naturaleza.

Objetivo: Definir valores de referencia hematológicos para dos especies de monos en Costa Rica.

Métodos: Durante el 2014 recolectamos muestras de sangre de monos aulladores de manto, *Alouatta palliata* (17 hembras, 18 machos) y monos capuchinos cariblanco, *Cebus imitator* (5 hembras, 7 machos) en siete localidades de la costa Pacífica de Costa Rica.

Resultados: Para ambas especies, los valores hematológicos fueron mayores en los machos, y las poblaciones de monos aulladores difirieron significativamente con excepción de las plaquetas.

Conclusiones: Estos valores hematológicos, que difieren según el sexo y la localidad, ayudarán a evaluar el estado de salud de estas poblaciones de primates neotropicales.

Palabras clave: primates neotropicales; mono aullador de manto; mono capuchino cariblanco; hematología; Costa Rica.

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