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Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*): A comparative study

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Abstract

Background: Evaluation of blood parameters in captive non-human primates (NHPs) is crucial for monitoring their health and ensuring that their environment meets their physiological requirements.

Methods: We performed hemogram, serum biochemistry, and parasitological exams in 20 howler monkeys and 21 capuchin monkeys.

Results: In both species, over 50% of the individuals presented at least one parasite. There was a negative effect of age on red blood cell (RBC), white blood cell, platelets, total protein, globulin, and alkaline phosphatase, and a positive effect on the A:G ratio, gamma-glutamyl transferase, and mean platelet volume (MPV). Capuchin monkeys presented the highest platelets and alanine aminotransferase (ALT) values and howler monkeys presented the highest MPV, aspartate aminotransferase, ALT, amylase, glucose, bilirubin, and triglycerides values. We observed an interaction between species and sex on RBC, Htc, mean corpuscular hemoglobin concentration, and cholesterol.

Conclusions: Species differences found in blood parameters may reflect differences in physiological adaptations associated with ecological and morphological traits and are clinically relevant for evaluating animal health and the suitability of breeding programs.

KEYWORDS
hemogram, non-human primates, platyrrhines, serum chemistry

1 | INTRODUCTION

Non-human primates (NHPs) have various behavioral and physiological similarities to humans and are useful in evolutionary studies and in biomedical research.[1] Among platyrrhine primates, capuchin monkeys (genus *Sapajus*) stand out in pharmacology and neuroscience studies,[2,3] while howler monkeys (genus *Alouatta*) have been used in studies on infectious and parasitic diseases.[4,5]

Although both species have sexual dimorphism,[6] they differ in average body mass (~5 kg for females and ~7.8 kg for male howler monkeys; ~3 kg for females and ~4 kg for male capuchins),[7] the average lifespan in captivity (26 years for howlers; up to 50 years for capuchins),[8,9] and diet. Howler monkeys are mainly folivores, with a diet rich in young leaves, shoots, buds, and different types of fiber and supplemented with fruits, seeds, and insects.[10–12] In contrast, capuchin monkeys have greater dietary diversity, with a diet composed mainly of fruits and insects, but can include larvae, seeds, roots, fossorial arthropods, small vertebrates, and eggs.[13–15] Considering that species have evolved specific physiological and morphological adaptations to different ecological niches, breeding programs must consider these differences when designing husbandry protocols for each species and conduct regular evaluation of physiological parameters to monitor their health and adaptability to captive conditions.

In this context, hematological and biochemical evaluations are standard laboratory tests in every animal facility and have been described in many platyrrhines such as capuchin monkeys,[16–19] howler monkeys,[20–22] owl monkeys (*Aotus azarae infulatus*),[23] squirrel monkeys (*Saimiri collinsi*),[24] black-tufted marmoset (*Callithrix penicillate*),[25,26] and spider monkeys (*Ateles geoffroy*).[27] However, these parameters can vary with sex, age, parasites, or if individuals are reared under different environmental conditions, which can interfere with the results.[28]

In our recent study, we investigated the effect of age and sex in kidney morphology and function, as well as differences in red blood cell (RBC) count. We found a higher absolute kidney volume in howlers, but higher relative kidney volume and RBC in capuchin monkeys. We also found a negative relationship between age and RBC in both species and a decrease in creatinine with age only in capuchins, suggesting that intra- and inter-specific factors can alter animal physiology and may illustrate differences in metabolic demands, the aging process, and general life strategies between these species.[29]

The aim of this study was to extend these analyses to compare the hemogram and serum biochemistry in captive howler and capuchin monkeys, considering the potential effect of species, age, sex, and the presence of intestinal parasites on blood parameters.

2 | METHODS

2.1 | Humane care guidelines

The experimental project followed the guidelines of the Brazilian Council for the Control of Animal Experimentation–Ministry of Science and Technology (CONCEA-MCT), and it was approved by the Ethics Committee for the Use of Animals (CEUA nos. 43/2019 and 24/2021) of the Institute Evandro Chagas (IEC), Ananindeua, Pará, Brazil and by the Biodiversity Authorization and Information System of the Chico Mendes Institute of Biodiversity (Sisbio/ICMBio, protocol 38529).

2.2 | Subjects

The subjects were 20 howler monkeys (*Alouatta caraya*—11 females and nine males), with a mean ± standard deviation (SD) body mass of 5.08 ± 3.48 kg (0.9–14.1 kg) and age range between 6 months and 26 years, and 21 capuchin monkeys (*Sapajus apella*—11 females and 10 males), with a mean ± SD body mass of 2.12 ± 0.79 kg (0.9–3.95 kg) and age range between 7 months and 21 years.

The animals were housed at the breeding colony of the National Primate Center (Centro National de Primatas–CENP), located at Ananindeua, Pará, Brazil (1°38′26″, 48°38′22″). We identified each animal by a three-letter code tattooed on the inner right thigh and a microchip placed in the interscapular area. All primate colonies at CENP are submitted to annual health screenings, which include physical examination, hemogram, and biochemical tests, in addition to deworming treatment. None of the animals used in this study had a history of infectious diseases as per their last health screening (2 months before data collection).

2.3 | Husbandry

All individuals lived in family groups of up to 10 individuals. They were kept in sheds and positioned in a north–south orientation to receive ≤12 h of natural light, in enclosures of dimensions 3.75 m × 2.2 m × 2.4 m (howler monkeys), and 3.85 m × 2.6 m × 2.5 m (capuchin monkeys). The enclosures had external and internal water bottles and multiple bowls for food provisioning. The animals were fed according to CENP's standard management practices. Their diet contained different types of fruits and vegetables, eggs, and commercial primate food with 18% crude protein (Cebidae P18 Megazoo, portion Megazoo). We also provided daily supplements of amino acids, vitamins, macro, and micro minerals, and 0.5 g of Aminomix Pet® (Vetnil Ind. Veterinary Products Ltda) per kg of body mass. Water was offered ad libitum.

2.4 | Fecal sample collection

We collected one fecal sample per animal for fecal parasitology tests shortly after defecation. The samples were stored in sterilized plastic containers labeled with an individual ID. Parasitology tests were performed according to a standard protocol established by CENP's parasitology laboratory, using direct examination techniques, as well as flotation and sedimentation.[30,31]

2.5 | Capture and blood collection

Following an 8-h fasting period, the animals were contained physically with the aid of nets, and chemically by intramuscular administration of a combination of ketamine hydrochloride (5 mg/kg), dexmedetomidine (0.01 mg/kg), and midazolam (0.2 mg/kg). With the animal contained, we collected between 2 and 3 mL of blood from the femoral vein with sterile syringes and needles (14–21G, depending on the species and age of the animal). Half of the sample was transferred to a tube containing ethylenediaminetetraacetic acid (EDTA) for hemogram and the other half was transferred to a tube without anticoagulants for clinical chemistry.

2.6 | Laboratory tests

The cell blood count was performed with an MS4+ blood analyzer (Melet Schloesing GmbH Central & Eastern Europe company, Sudstadtzentrum 1, Top 8) to determine RBC count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, differential WBC count (segmented, lymphocytes, monocytes, eosinophils, basophils), platelets, and mean platelet volume (MPV). Biochemistry tests were performed on sistema Vitros DTSC II, DT60, and DTE2 (Johnson & Johnson Medical Argentina), to evaluate the total protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin (BIL), glucose, amylase, lipase, triglycerides, and cholesterol. Globulin value was calculated by subtracting albumin from TP values, then albumin/globulin ratio (A:G ratio) was calculated. All values were reported and followed by the respective reference values from the literature. Kidney function (urea, and creatinine) was evaluated and discussed in our previous study.[29]

2.7 | Statistical analysis

All statistical tests were performed in R software (3.3.0). General linear models (GLMs) were used to test the effects of age, sex, species, and parasitism on blood parameters. First, the possibility of multicollinearity was excluded by calculating the variance inflation factor (VIF) with the "car" package. Since all factors had VIF < 2, none were considered problematic in the model. To assess the equality of variances of categorical fixed factors, we used Levene's test.[32] Models that showed non-normality of residuals were power-transformed using *boxcox* function from the package *MASS*.[33] However, the residuals for the models MCV and MCH were not normally distributed even after transformation. Thus, they were excluded from the statistical analyses, but we reported their descriptive statistics.

We built seven models for blood count parameters (predictor variables: RBC, Hb, Hct, MCHC, WBC, platelets, and MPV). The differential WBC data were not submitted to statistical analyses as some of these parameters were zero-inflated but given the importance of the data in interpreting alterations in WBC count, we calculated their mean ± SD per species, sex, and age and included the data in the results. We also built 14 models for biochemical parameters, (predictor variables: TP, ALT, ASP, amylase, GGT, lipase, glucose, ALP, bilirubin, albumin, globulin, A:G ratio, triglycerides, and cholesterol). For all parameters, we initially included as fixed factors: age, body mass, species, sex, and parasitism (individuals infected with parasites were considered as "positive" and individuals that were not infected with parasites were considered as "negative"), and their interactions. Following Burnham and Anderson,[34] we sequentially removed fixed factors to select the model with the lowest Akaike information criterion with correction for small sample sizes (AICc). If AICc difference between the two models (ΔAICc) was less than 2, both models were discussed. We reported the mean ± (SD) and range (minimum–maximum) values for all parameters described in this study by species, sex, and age (Tables S1 and S2), and by sex only to compare our data with reference values reported in previous studies (Tables S3 and S4). Due to the limited data available in the literature, we included references that matched our study subjects at the genus level (*Alouatta*[21,22,35,36] and *Sapajus*[16,37]).

3 | RESULTS

3.1 | Parasitology tests

We were not able to collect fecal samples from infant howler monkeys ($n = 3$) due to their constant close contact with the mother. Among the remaining individuals of this species, we found that 47% (8/17) were positive for parasites. Specifically, we detected *Giardia lamblia* in 22% (4/17), *Entamoeba coli* in 17.6% (3/17), *Pentatrichomonas hominis* in 11.7% (2/17), and *Strongyloides stercoralis* in 5.8% (1/17). In capuchin monkeys, a total of 66.7% (14/21) individuals were parasitized, with *Ancylostoma* spp. in 57.1% (12/21), *S. stercoralis* in 47.6% (10/21), *P. hominis* in 9.5% (2/21), and *Entamoeba histolytica* in 4.7% (1/21).

3.2 | Hemogram and serum chemistry exams

All GLM models are shown in detail in Tables 1 and 2 (hemogram) and (serum chemistry).

The models including RBC as a predictor revealed a significant negative effect of age, and an interaction between sex and species, with a lower value in male capuchins than females, and the opposite effect in howler monkeys. For models including Hb, Hct, and MCHC as a predictor, we found an interaction between sex and species similar to the effect found for RBC, with lower values in male capuchins compared to females, but the opposite trend in howler monkeys, but no effect of age or parasitism (Figure 1).

For WBC, we found a significant negative effect of age and interaction between parasitism and species, in which positive capuchin monkeys had higher values than negative conspecifics, but the opposite trend in howler monkeys (Figure 2). Based on this result, we calculated the mean ± SD values of the differential WBC count by species and parasite condition (Table 3), which shows that eosinophils were higher in positive capuchin monkeys but fell below or undetectable levels in negative capuchin monkeys and in all howler monkeys studied. Positive capuchin monkeys also presented an increase in segmented, lymphocytes, monocytes, and basophils in comparison with negative conspecifics and any howler monkey group, whereas positive and negative howler monkeys presented similar results.

The model for platelets showed a significant effect of species, with a higher platelet count in capuchins than in howler monkeys.

TABLE 1 Generalized linear models (GLM) investigating the effects of species, sex, age group, and presence of parasites in hematological parameters in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Hematologic parameter	Effect	Estimate	Standard error	*Z* value	*p*-value
Red blood cells (×10^6 per mm)	Intercept	4.43	0.19	23.34	<0.001
	Age	−0.03	0.01	−2.46	0.02
	Species_sex (*Sapajus apella*_male)	−0.06	0.31	−2.10	0.04
Hemoglobin (g/dL)	Intercept	14.32	0.51	27.90	<0.001
	Species_sex (*Sapajus apella*_male)	−2.18	0.85	−2.57	0.01
Hematocrit (%)	Intercept	42.62	1.60	26.77	<0.001
	Species_sex (*Sapajus apella*_male)	−6.67	2.63	−2.53	0.02
Mean corpuscular hemoglobin concentration (%)	Intercept	13.80	0.42	32.37	<0.001
	Species_Sex (*Sapajus apella*_male)	−1.84	0.88	−2.08	0.04
White blood cells (×10^3 per mm)	Intercept	209.12	9.82	21.28	<0.001
	Age	−1.18	0.56	−2.11	0.04
	Parasites_specie (positive_*Sapajus apella*)	55.30	16.60	3.33	0.002
Platelets (×10^3 per mm)	Intercept	216.60	16.33	13.26	<0.001
	Species (*Sapajus apella*)	31.46	15.45	−2.07	0.04
	Sex (male)	−31.24	15.21	−2.05	0.04
	Age	−4.50	1.07	−4.20	<0.001
Mean platelet volume (%)	Intercept	12.83	0.53	24.36	<0.001
	Species (*Sapajus apella*)	−2.76	0.50	−5.49	<0.001
	Age	0.11	0.03	3.07	0.004

We also observed an effect of sex, with lower platelet count in males compared to females, as well as a significant negative effect of age. The model for MPV revealed an effect of species and age, with lower MPV in capuchin monkeys than in howler monkeys and a positive relationship with age (Figure 3).

The models including TP and globulin as response variables showed a negative effect of age and an effect of parasitism, with higher TP and globulin in positive animals. However, there was an opposite trend for the A:G ratio as a response variable, with a positive relationship with age and lower values in parasitized animals (Figure 4).

In hepatic enzyme models, the AST model showed an effect of species, with lower values in capuchins than in howler monkeys. The ALT model, however, showed the opposite effect, with significantly higher values in capuchins than in howler monkeys. In addition, there was an effect of parasitism, with lower ALT in positive than negative animals (Figure 5). The GGT model revealed a positive relationship with age, and the ALP model revealed the opposite effect, with a significant negative effect of age. In the bilirubin model, there was an effect of species, sex, and the presence of parasites, with higher BIL in howler monkeys, males, and positive animals, respectively (Figure 6).

Both models for amylase and glucose showed an effect of species, with lower levels in capuchins compared to howler monkeys (Figure 7). In relation to lipidogram parameters, the model including cholesterol as a predictor showed an interaction between species and sex, with lower levels in male capuchin monkeys compared to female conspecifics, but the opposite trend in howler monkeys. In the triglyceride model, we found an effect of species, with lower levels in capuchins than in howler monkeys (Figure 8).

For lipase and albumin, the models including fixed factors did not differ from the null model, demonstrating that age, sex, parasitism, and species did not interfere with these parameters.

4 | DISCUSSION

Research centers that maintain NHP must adhere to safety and sanitation protocols to ensure animal health, and quality of research, and to avoid pathogen transmission between animals and keepers.[38] In the present study, parasites were present at an incidence rate above 50% in both NHP species investigated. Thus, periodical clinical examination, coproparasitological examinations, and medical therapy are essential to diagnose and control helminth dissemination in captivity, especially in the Amazon, where climatic conditions favor pathogen multiplication.[39]

Among the parasites found in this study, *Giardia lamblia* is an intestinal parasite commonly transmitted by water and infecting humans, birds, marsupials, small rodents, and carnivores.[40,41] Its main hosts are NHP, with several cases reported in platyrrhine species, including squirrel monkeys (*Saimiri sciureus*), spider monkeys (*Ateles fusciceps*), cotton-top tamarins (*Saguinus oedipus*), and howler monkeys (*Alouatta* spp.).[42,43] Captive primates generally have higher infection rates compared to free-ranging animals, as the confined environment allows *Giardia* cysts to spread more easily.[44,45] The genus *Entamoeba* is composed of protozoa with high zoonotic potential

TABLE 2 Generalized linear models (GLM) investigating the effects of species, sex, age group, and presence of parasites in serum biochemistry parameters in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Serum biochemistry	Effect	Estimate	Standard error	*t*-value	*p*-value
Total proteins (g/dL)	Intercept	8.24	0.30	27.23	<0.001
	Age	−0.10	0.02	−5.17	<0.001
	Parasites (positive)	1.05	0.29	3.59	0.001
Albumin (null model)	Intercept	16.93	0.49	34.37	<0.001
Globulin	Intercept	4.36	0.19	23.43	<0.001
	Age	−0.09	0.01	−5.74	<0.001
	Parasites (positive)	1.07	0.23	4.66	<0.001
Albumin/globulin ratio	Intercept	0.98	0.05	20.43	<0.001
	Age	0.02	0.004	5.08	<0.001
	Parasites (positive)	−0.22	0.06	−3.93	<0.001
AST (U/L)	Intercept	2.47	0.02	93.18	<0.001
	Species (*Sapajus apella*)	- 0.19	0.02	−7.61	<0.001
ALT (U/L)	Intercept	15.52	3.06	5.07	<0.001
	Species (*Sapajus apella*)	24.80	2.90	8.53	<0.001
	Parasites (positive)	−7.70	2.95	−2.61	0.014
Amylase (U/dL)	Intercept	346.06	28.09	12.32	<0.001
	Species (*Sapajus apella*)	−149.88	25.89	−5.79	<0.001
GGT (U/L)	Intercept	-9.13×10^{-4}	1.28×10^{-4}	−7.15	<0.001
	Age	2.50×10^{-5}	8.24×10^{-6}	3.03	0.005
Lipase (U/L) (null model)	Intercept	9.38	1.13	8.28	<0.001
Glucose (mg/dL)	Intercept	124.51	12.19	10.21	<0.001
	Species (*Sapajus apella*)	−38.43	11.57	−3.32	0.002
Bilirubin (mg/dL)	Intercept	−31.47	5.89	−5.34	<0.001
	Species (*Sapajus apella*)	−25.23	5.93	−4.25	<0.001
	Sex (male)	15.79	6.12	2.58	0.02
	Parasites (positive)	15.30	6.18	2.48	0.02
	Intercept	135.94	12.50	10.83	<0.001
Cholesterol (mg/dL)	Species_sex (*Sapajus apella*_male)	−52.88	22.63	−2.33	0.02
	Intercept	4.42	0.12	36.92	<0.001
Triglycerides (mg/dL)	Species (*Sapajus apella*)	−0.64	0.14	−4.62	<0.001
ALP (U/L)	Intercept	−0.1	0.07	−1.40	0.174
	Age	−0.01	0.00	−3.27	0.003

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

that cause intestinal diseases and extraintestinal abscesses, with *E. coli* being one of the species most excreted by NHPs.[46,47] The increase in primates infected by this protozoan is usually related to the greater resistance of uninucleated cysts to amoebicidal drugs and the lack of treatment, as they rarely present clinical manifestations of intestinal lesions.[48,49]

In capuchin monkeys, the parasites reported were *Ancylostoma* spp., *Strongyloides stercoralis*, *Pentatrichomonas homini*, and *Entamoeba histolytica*. This genus has infections that may be prevalent due to their omnivorous diet, frequent contact with soil, large group sizes, and active social behavior.[50] The genus *Ancylostoma* is one of the most common in NHPs, along with strongylida, and the infection occurs by transcutaneous transmission by larvae that migrate to the gastrointestinal tract.[51] In captive primates, the incidence of this parasite has been associated with poor hygienic conditions. Thus, keeping the animals dewormed and in clean cages with filtered water and balanced food reduces contamination.[52,53] *E. histolytica* occurs in several species of NHPs, being more common in platyrrhine species because they are more sensitive. The strains are identical to humans strains and can be transmitted via the fecal–oral route through food and water contaminated with cysts.[54]

In this study, *S. stercoralis* and *P. hominis* were found in both species. *Strongyloididae* is one of the most prevalent groups of

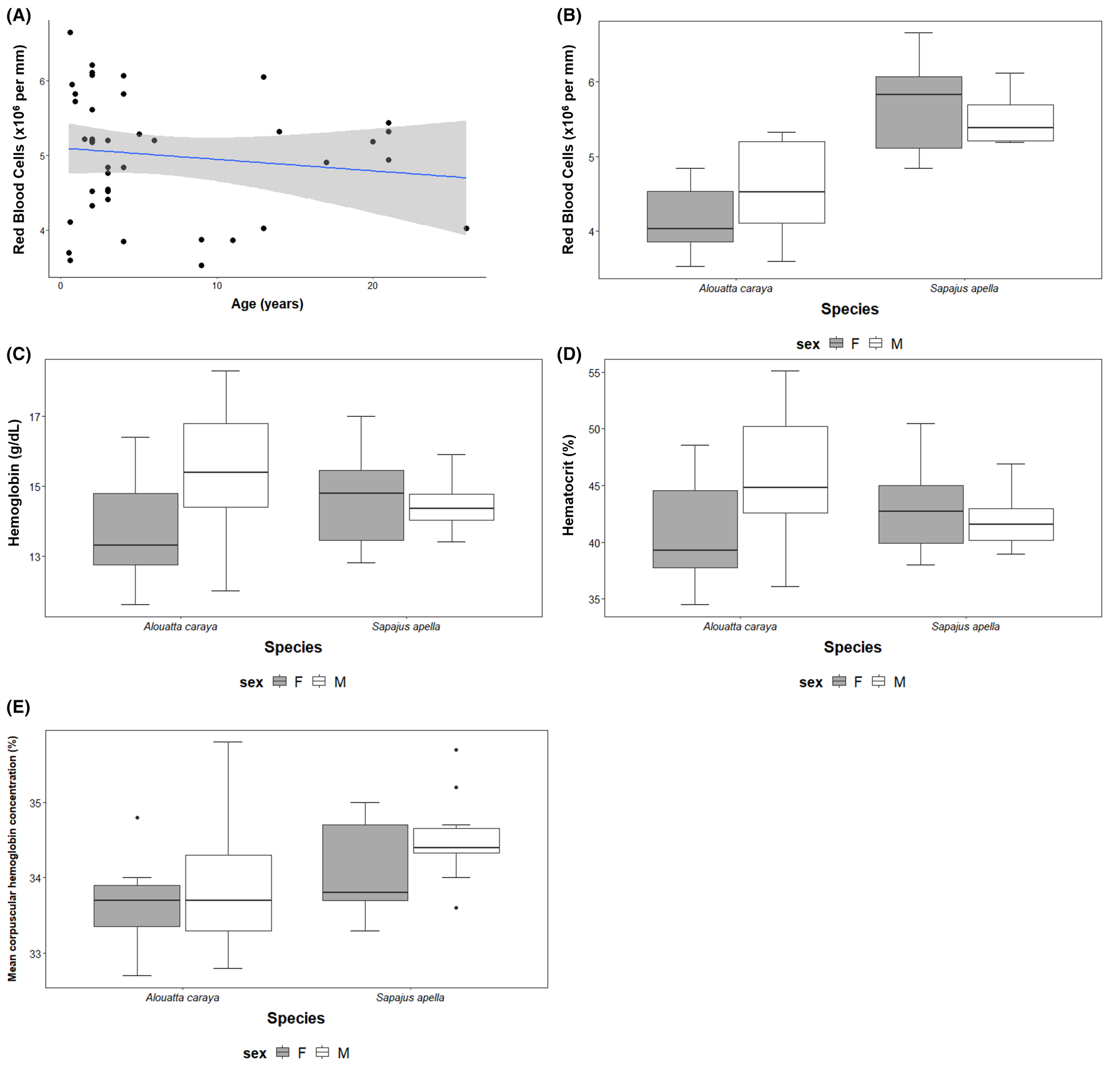

FIGURE 1 Negative effect of age in red blood cells (A); interaction between species and sex (F—female; M—male) in red blood cells (B), hemoglobin (C), hematocrit (D) and mean corpuscular hemoglobin concentration (E), in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

parasites,[51] found in hot and humid climates, and can infect hosts through skin penetration or when ingested.[55,56] This parasite reproduces asexually in the host's intestinal wall, contributing to high rates of infection.[57,58] *P. hominis* is a trichomonad that is commonly found in the intestinal tract of domestic animals and primates and may pose a risk of zoonotic and anthroponotic transmission.[59,60] The main form of transmission is the fecal–oral route, through the ingestion of contaminated food and water or through direct contact from one host to another, as the flagellated form does not survive long in the environment.[60,61] This protozoan is considered a non-pathogenic opportunistic agent and is generally not the main agent of intestinal lesions in NHPs.[62]

4.1 | Blood parameters

The age effect on RBC count in both species, suggests a decline in hematopoiesis in older individuals. Likewise, Núñez et al.[63] and Ferreira et al.[64] found higher values in young animals compared to adult capuchin monkeys (*Sapajus apella* and *S. libidinosus*). These results could be associated with changes in erythroid progenitor cells, in the cell hematopoietic microenvironment, and in humoral changes.[65] In addition, the bone marrow of young animals and humans has a higher percentage of red bone marrow, which is hematopoietically more active than yellow bone; this last one contains more adipose tissue and is more abundant in adults.[66]

The interaction between sex and species on RBC count, Hb, Hct, and MCHC revealed that while male howler monkeys had higher values than females for these parameters, female capuchin monkeys had higher values than their male conspecifics. However, the interaction does not indicate whether these differences are significant or not, so it is possible that the interaction was a product of our sample size. The sex differences observed in howler monkeys are consistent with previous reports in platyrrhine species, including other howler monkey species,[21,22,67] capuchin monkeys,[16,63,64,68,69] owl monkeys,[23] squirrel monkeys,[24] black-tufted marmoset,[25,26] spider monkeys,[27] and humans.[70] These sex differences have been associated with the stimulatory effect of testosterone on erythropoiesis, and the inhibitory effect of estrogen[16,37,70,71] but also related to genetic differences, such as the difference between males and females in erythropoietin gene and its receptor.[72]

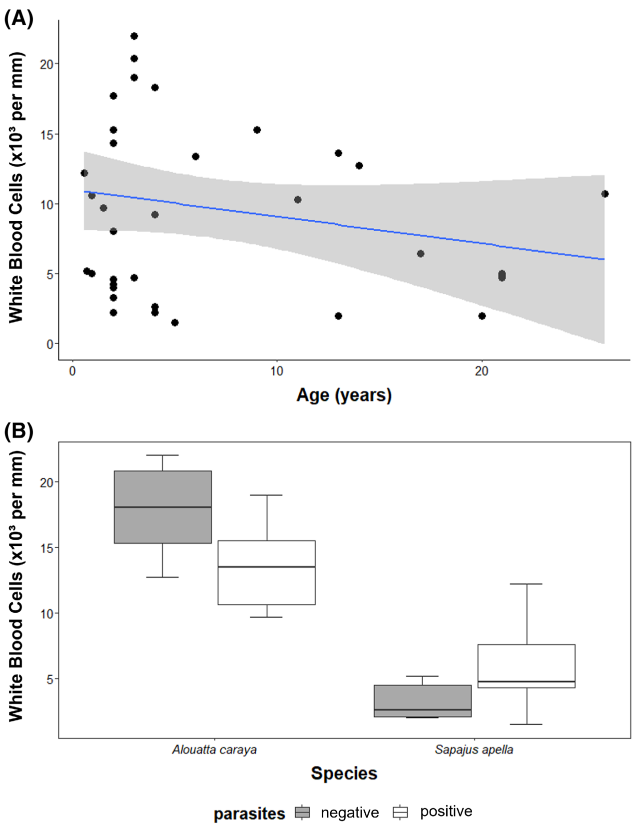

FIGURE 2 Negative effect of age (A) and interaction between species and parasite presence (B) in white blood cells in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

The negative effect of age on WBC in our study was consistent with a previous study in capuchin monkeys,[64] in which juveniles had higher WBC compared to adults. In infants, the bone marrow is hematopoietically active in all bones, whereas in adults and elderly, only the sternum, femur, and flat bones are hematopoietically active.[73,74] Another possibility is the major propensity of young animals to release epinephrine due to excitement or fear, causing neutrophilia, eosinophilia, and lymphocytosis due to leukocyte mobilization.[75,76]

We also observed an interaction between parasitism and species on WBC, in which higher values of WBC were found in positive capuchin monkeys but the opposite trend in howler monkeys. Changes in WBC depend on parasite load, the intensity and pathogeny of infection, and immune response. *Strongyloides stercoralis* is one of the most clinically important pathogenic species in NHP.[58] This parasite species was found in 10 capuchin monkeys but only in one howler monkey in the present study. Therefore, the interaction found in this result may have been a product of different parasite species eliciting different immune responses. Interestingly, we found that in the differential WBC, eosinophils were elevated only in positive, but not in negative, capuchin monkeys, nor in any howler monkey studied. Eosinophilia is commonly associated with parasite infection,[77] and our results for differential WBC suggest that the degree of pathogenicity of parasitosis may vary by both parasite and host species. This result highlights the importance of analyzing differential WBC count to determine and establish the diagnosis of each clinical condition. Although the WBC values were in accordance with the reference values available in the literature for capuchins,[16] were not for howler monkeys[21] (Table S3), thus we must consider intraspecific variations in WBC due to antigenic stimulation, stress during animal handling, and anesthesia.[21,77]

In this study, males had significantly lower platelet counts than females in both species, which was similar to results described recently in spider monkeys.[78] Previous studies, although without significant difference, reported lower values for platelets in males in other platyrrhine species such as howler monkeys,[21,35,67] capuchin monkeys,[19,69,79] spider monkeys,[27] and in catarrhines including vervet monkeys (*Chlorocebus aethiops sabaeus*)[80] and long-tailed macaques (*Macaca fascicularis*) and rhesus monkeys (*M. mulatta*).[81] Furthermore, in humans, the higher platelets observed in women[82,83]

TABLE 3 Differential WBC count (mean ± SD) by species and parasite condition in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Parameter ($\times 10^3$ per mm^3)	*Alouatta caraya*		*Sapajus apella*	
	Negative (*n* = 9)	Positive (*n* = 8)	Negative (*n* = 7)	Positive (*n* = 14)
Segmented	8.93 ± 4.13	5.64 ± 3.34	1.57 ± 0.96	3.50 ± 2.24
Lymphocytes	7.02 ± 1.58	6.83 ± 2.48	1.37 ± 0.69	1.69 ± 1.07
Monocytes	1.05 ± 0.36	1.10 ± 0.39	0.26 ± 0.24	0.40 ± 0.39
Eosinophils	0	0	0.003 ± 0.007	0.11 ± 0.15
Basophils	0.2 ± 0.05	0.17 ± 0.05	0.04 ± 0.005	0.13 ± 0.14

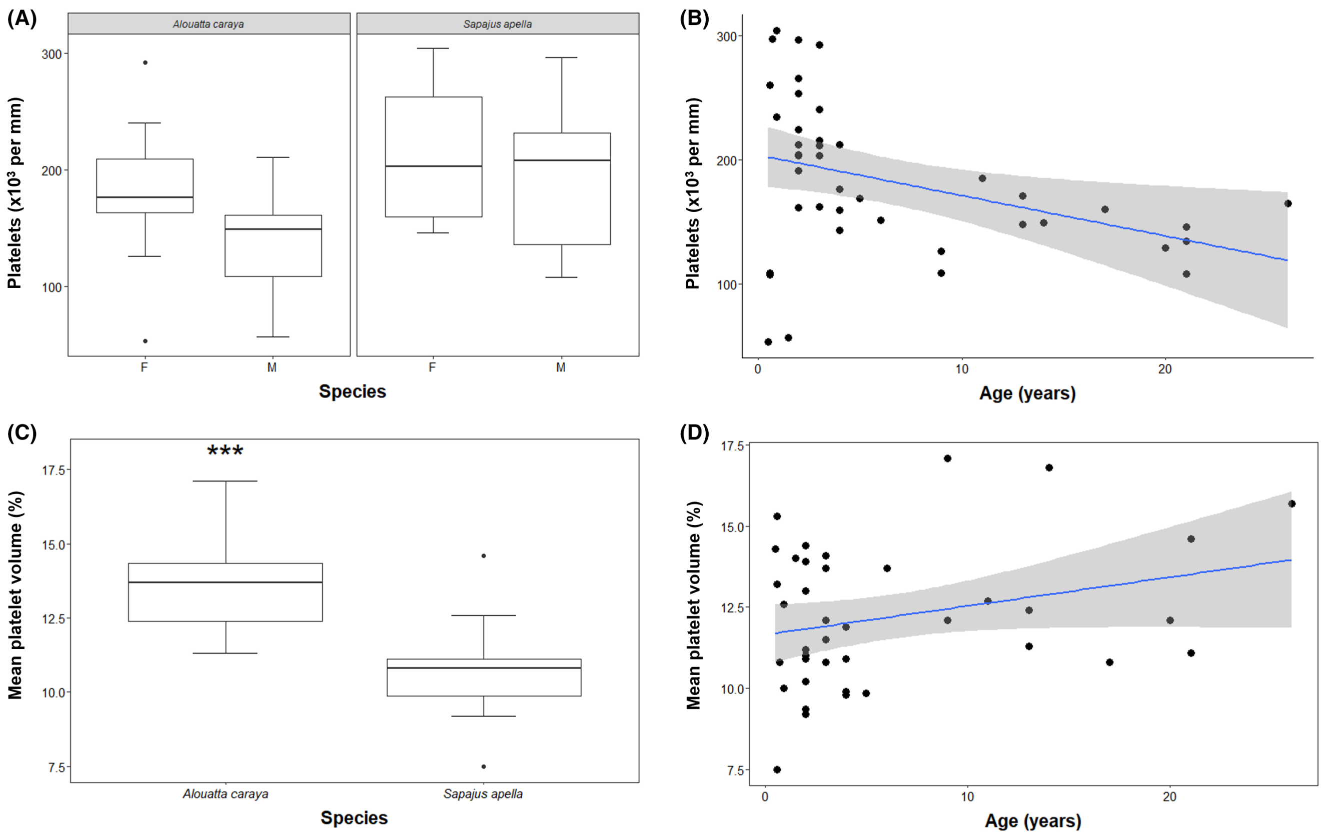

FIGURE 3 Effect of species (A) and age (B) in platelet count; effect of species (C) and age (D) in mean platelet volume in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

can be associated with the importance of platelet aggregation as possibly a mechanism to prevent heavy menstrual bleeding.[84] Since menstrual cycles have been described in both howler and capuchin monkeys,[85,86] this result may indicate an early adaptation in female primates.

The higher platelet count for capuchin monkeys found in our study corroborates with other studies in captivity and in wild.[16,18,19,21,22,35,63,79,87] We also found a higher RBC count in capuchin monkeys that was related to the higher metabolic demands in capuchin monkeys,[29] and platelet count seems to follow the same trend in these species.

We also observed a negative effect of age on platelet count. Other studies showed the same trend, though not significant, in capuchin monkeys,[16,63] marmosets (*Callithrix* sp.),[26] owl monkeys,[23] and spider monkeys.[27] This differs from one study in humans, in which the platelets had a significant positive correlation with age, though the authors highlighted that the impact of age seemed to be of minor relevance when compared with other factors such as sex and MPV.[83] Another possible explanation is the interaction of platelets and WBC by forming platelet–WBC aggregates in inflammatory responses.[88] Considering that age had a negative effect on WBC, it is possible that platelets followed the same pattern due to higher inflammatory responses in younger individuals.

MPV was significantly lower in capuchin monkeys. MPV is a possible determinant of platelet function and aggregability, and large platelets are more active than normal-sized platelets[89,90]; these results can be explained by the fact that howler monkeys presented a lower value of platelets compared to capuchin monkeys in this and in previous studies.[21,22,35,79] The higher value for MPV can be a compensatory mechanism in this species for a high platelet volume as a trade-off to a low platelet count. This is consistent with the positive effect of age on MPV, which is the opposite of what we found for platelet count. In normal physiological conditions, MPV is inversely proportional to the platelet count, which is associated with hemostatic maintenance and preservation of constant platelet mass[91]; thus, an increase in the production of platelets is accompanied by a reduction in their mean volume.[92]

4.2 | Biochemistry tests

The negative effect of age on TP and globulin values observed in our study was similarly described by Rodriguez et al.[93] and Scobar[94] in woolly monkeys (*Lagothrix lagotricha*), in which juveniles had higher TP values than sub-adults, suggesting that younger animals have higher globulin concentrations due to greater demand during growth. These results differ from those observed in chimpanzees (*Pan troglodytes*), which experienced an increase in globulin and a decrease in albumin with age.[95] In contrast, we did not observe any significant effect on albumin values in our study. We also observed an increase

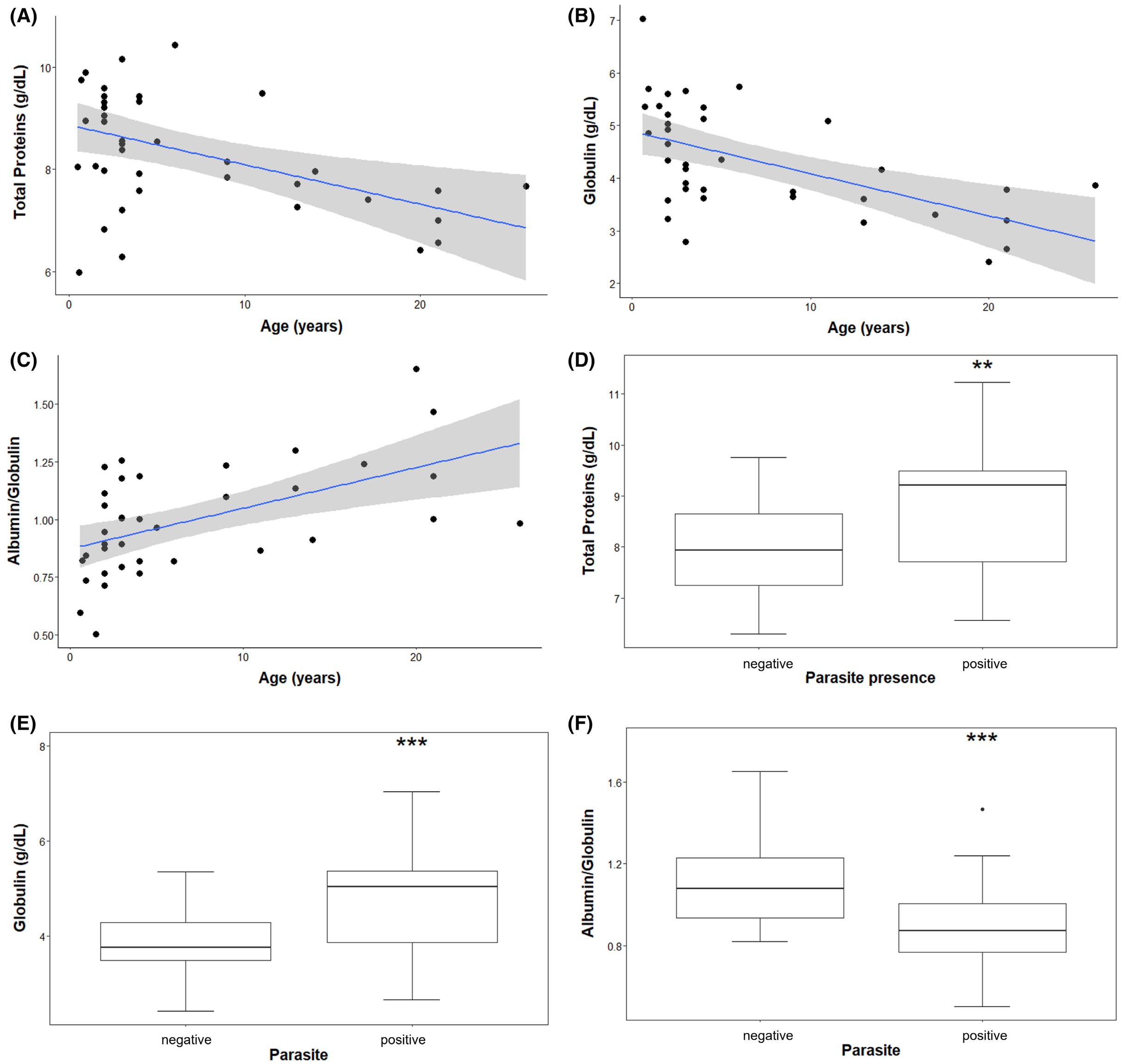

FIGURE 4 Negative effect of age in total protein (A), globulin (B) and albumin: globulin ratio (C); effect of parasite presence in total proteins (D), globulin (E), and albumin: globulin ratio (F) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

in TP and globulin in parasitized animals. Some liver proteins are carriers of molecules to promote enzymatic activity or participate in the innate immune response and are considered one of the earliest markers for any pathologic process or disease.[96] The increase in globulin in positive animals can be associated with the host's innate immune system in response to parasite infection,[97,98] as globulins are considered positive acute phase proteins (APPs), whereas albumin is considered a negative APP that decreases in infection cases.[96] Even though no changes were observed in albumin with parasitosis, we found that the A:G ratio was lower in positive animals, suggesting that the A:G ratio is a better index than albumin alone to diagnose parasite infection due to its higher sensitivity.

In relation to liver enzymes, we found lower AST levels in capuchin monkeys than in howler monkeys, which is consistent with results reported previously in these species.[16,22,35,63,87] In primates, AST is found in the mitochondria of the hepatocyte, and an elevation in its activity can be associated with liver damage.[99,100] Other sources of AST are the heart, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and erythrocytes.[101] However, none of the animals in our study presented alterations related to liver or muscular/cardiac function on clinical/ultrasound tests nor historic therapy with hepatotoxic drugs. Another reason for the increase in AST is related to muscular activity and degeneration. Muscles present two main types of fibers: Type I (slow twitch) and Type II (fast twitch). Type I fibers receive more glucose and oxygen than Type II and can compete for resources with the brain.[102] One study showed that larger-brained primates such as capuchin monkeys have fewer muscle fibers Type I than primates with smaller brains,[103] thus lower AST levels in capuchin monkeys may be a product of differences in muscle fiber. Considering that capuchin monkeys have a higher encephalization index than howler monkeys,[103,104] this result supports the expensive tissue hypothesis (ETH), aaa which postulates that an

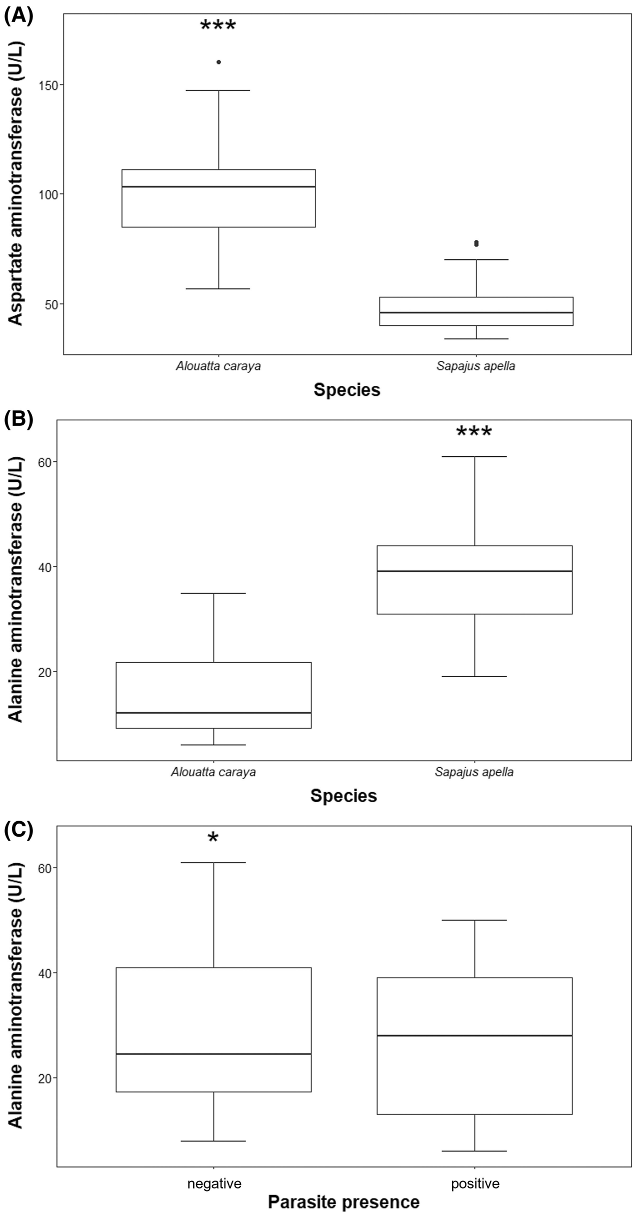

FIGURE 5 Effect of species in aspartate aminotransferase (A) and alanine aminotransferase (B); effect of parasite presence alanine aminotransferase (C) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

increase in the brain mass is compensated by a reduction in the mass of metabolically costly tissues such as the gastrointestinal tract[105] and skeletal muscle, though further comparative studies in other species are warranted to test this hypothesis.

In contrast with AST, we observed higher ALT levels in capuchin monkeys, which is similar when comparing the mean values reported in previous studies with these species.[16,18,21,22,35,63,87] The enzyme ALT is a transaminase hepato-specific in carnivores and has been useful as a marker of liver damage as it is released by injured hepatocytes.[102,106] We also observed that ALT was lower in parasitized animals in both species, but this result might not be clinically relevant, given the large variation in normal ALT values reported in previous studies.[16,21,37,69] Additionally, this effect may depend on parasite load and species, which were not evaluated in the present study.

In relation to GGT, we found a positive relationship with age, which is consistent with another study in capuchin monkeys.[16] Also, Núñez et al. (2008)[63] reported a higher value in adult capuchins compared to juveniles, though the difference was not significant, probably due to sample size. Interestingly, previous studies have found that GGT affects RBC integrity. Glutathione metabolism mediated by high concentrations of GGT can give rise to pro-oxidant substances when chelated transition metals are present. This results in the production of reactive oxygen species, which induces lipid peroxidation, and pore formation in the cell membranes of RBC, causing hemolysis.[107] This may explain our results on age decline in RBC count. Thus, GGT function is a promising biomarker of aging. Likewise, in humans, serum GGT has been reported as a remarkable predictor for a multitude of age-related diseases and chronic conditions such as liver disease and bile duct disorders, cardiovascular disease, metabolic syndrome, obesity, diabetes, and cancer,[108] which are linked to the presence of the enzyme GGT in bile ducts, kidneys, pancreas, and intestine.[101]

For ALP, the significant negative effect of age observed in our study was similar to previous reports in howler monkeys,[109] capuchin monkeys,[16] woolly monkeys,[93] and owl monkeys.[23] This enzyme is used for the evaluation of liver or bone diseases, given that more than 80% of serum ALP originates from these tissues.[101] Thus, higher serum activity in younger animals may be related to the increased metabolic activity of osteoblasts during bone development.[110,111]

For bilirubin, we observed that capuchin monkeys had lower levels than howler monkeys, and bilirubin was higher in males than females. The species effect was similar when comparing bilirubin levels in previous reports of these species[16,35] and the sex effect was also observed in woolly monkeys.[94] These results may be linked with differences observed in hemoglobin since 80% of bilirubin is made from the breakdown of hemoglobin in heme products released in senescent RBC.[112] Furthermore, a higher value was observed in parasitized animals in both species, which could indicate that the parasites present in the study affected the hepatobiliary system and that bilirubin may be an early marker of helminthic infestation. However, further analyses that account for parasite load and pathogenicity are needed to determine the clinical accuracy of bilirubin in the diagnosis of parasite infection.

Glucose and amylase were only affected by species, with lower values in capuchin monkeys than in howler monkeys. Glucose values found in our study differ from those reported in other studies in capuchins,[8,16,18] which are generally higher than those reported in howler monkeys.[22,35,67] Although hyperglycemia is associated with diabetes mellitus, is also observed in anesthetized animals (liver glycogen mobilization for circulation), and in pancreatitis,[113,114] which may be associated with metabolic and dietary differences between capuchins (omnivorous) and howler monkeys (folivorous).[115,116] The unexpected higher results in howler monkeys may be related to the low activity of this genus in captivity and the provisioning with fruits in captivity which leads to increased sugar intake and reduced

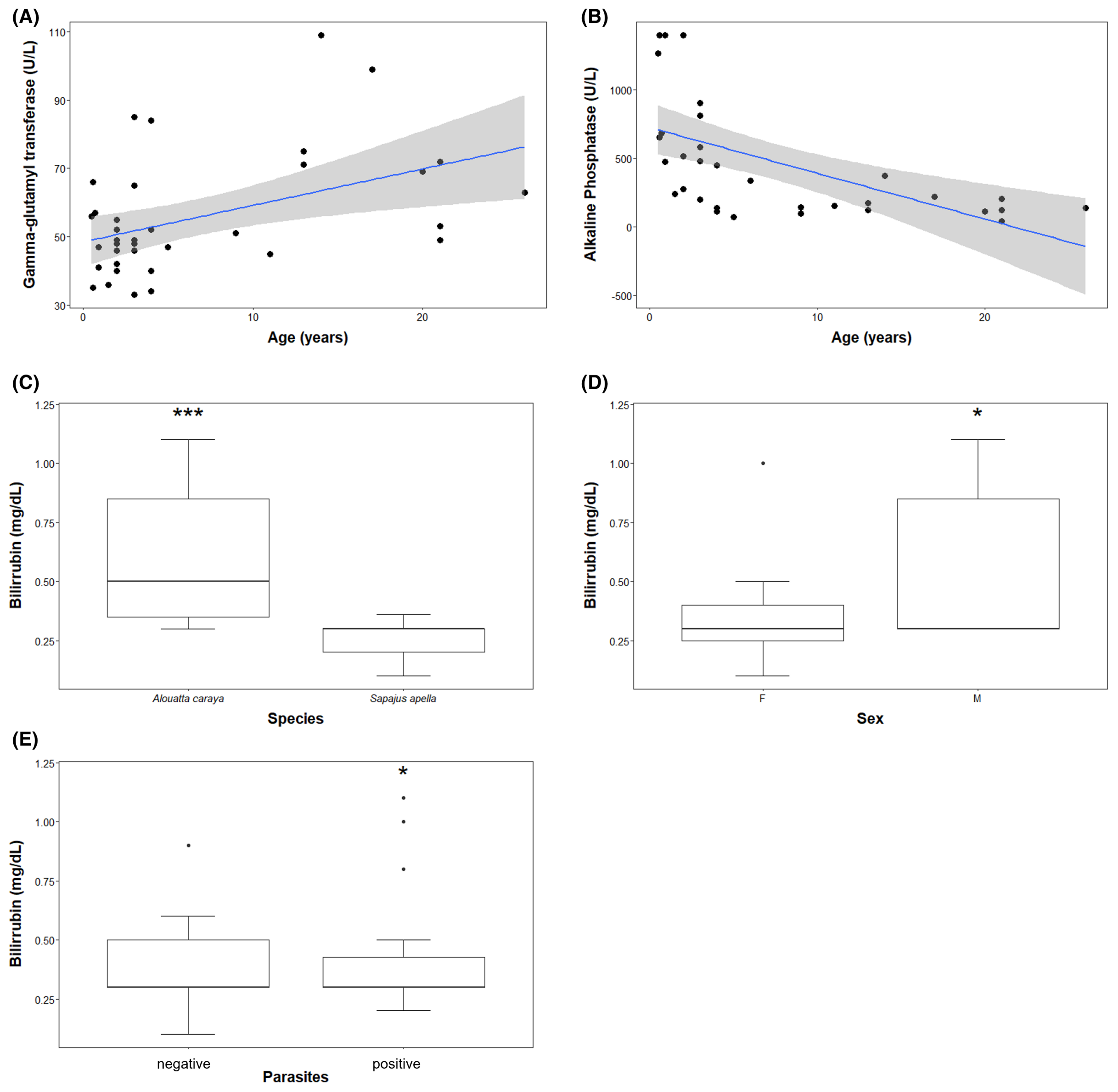

FIGURE 6 Effect of age in gamma-glutamyl transferase (A) and alkaline phosphatase (B); effect of species (C), sex (D), and parasite presence (E) in bilirubin in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

energetic expenditure, which predisposes to diseases such as diabetes. In fact, one howler monkey from the colony was excluded from this study because it presented a diagnosis of diabetes, which supports our hypothesis.

For amylase, the higher value in howlers than in capuchin monkeys is consistent with comparisons using data from other studies in these species.[16,35] Amylase is necessary for starch digestion in the small intestine due to its important function in hydrolyzing the glycosidic bonds in starch molecules and converting complex carbohydrates to simple sugars.[117,118] Furthermore, Campos et al. (2010)[119] reported an increase in amylase associated with acute necrotic pancreatitis in howler monkeys and associated this result with long-term inadequate nutritional management. Thus, considering the high values of amylase reported in our study and others in the literature, as well as glucose, it is important to monitor the pancreatic biomarkers in these species in order to avoid metabolic diseases, particularly in folivore species such as howler monkeys, and increment their diet with a wide variety of fiber sources, such as leafy greens and natural browse, to promote healthy natural gut microbiota and digestion.[115]

On lipidogram, we observed an interaction between species and sex for cholesterol, with lower cholesterol levels in male capuchins compared to females, but the opposite in howler monkeys. Cholesterol is found in cell membranes and is a precursor of bile acids and steroid hormones.[101] Our results for capuchins are in accordance with other studies in capuchin monkeys (*Cebus* spp.),[37] squirrel monkeys,[24] and humans.[120] Although capuchin monkeys have a sexual dimorphism in body mass in which males are heavier than females,[121] Edwards et al.[122] reported higher body fat percentages in females (21.2 ± 1.3%) compared to males (18.2 ± 1.8%), which can explain the higher cholesterol values in females observed in our

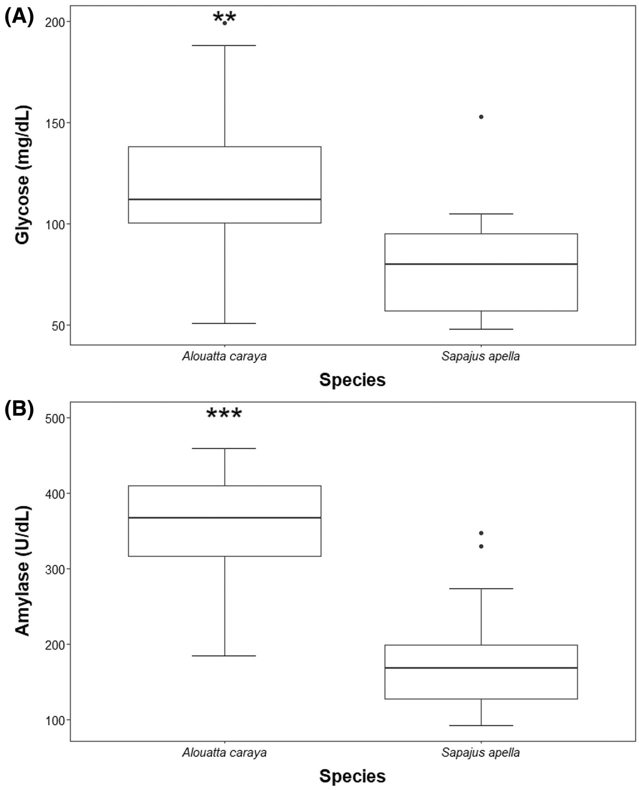

FIGURE 7 Effect of species in glucose (A) and amylase (B) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

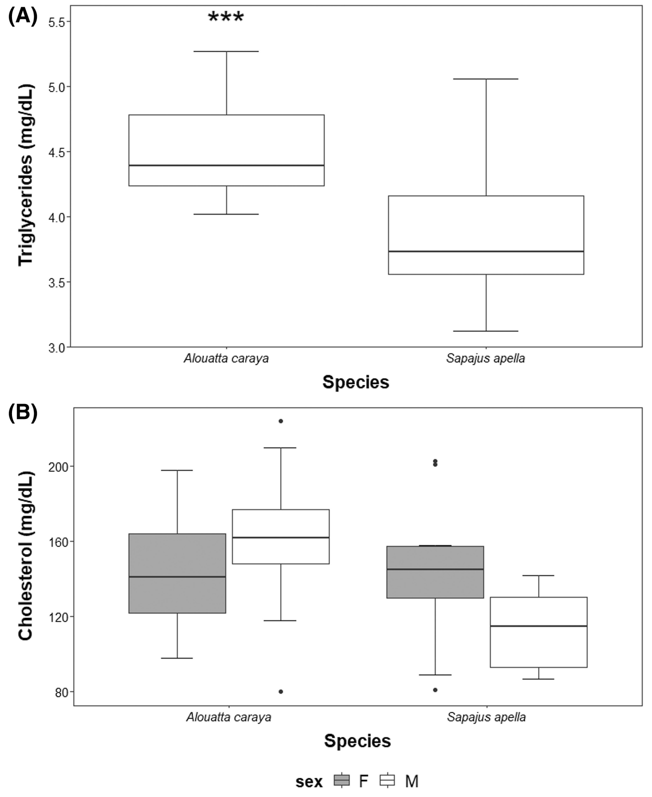

FIGURE 8 Effect of species in triglycerides (A) and interaction between species and sex (F—female; M—male) in cholesterol (B) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

study. Our results for howlers were similar to those of other studies in this species, where females had lower cholesterol levels compared to males.[68,79] Since howler monkeys also have sexual dimorphism in body mass as described in capuchin monkeys,[121] our results may suggest that males in this genus have higher fat percentages than females, but further studies are necessary to confirm this hypothesis.

Differences in fat percentages can also explain the lower triglyceride values in capuchins than in howler monkeys in the present study, which contrasts with previously reported data in these species.[35,37,87] Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat deposits in animals. They act as energy storage and transport energy from the small intestine and liver to peripheral tissues.[79,102,123] The body mass averages 2.12 ± 0.79 kg in capuchin monkeys and 5.08 ± 3.48 kg in howler monkeys, and this difference is greater when considering lean body mass.[124] Moreover, considering the slow metabolism of howlers when compared to capuchin monkeys,[125,126] the effect of captivity on reduced energy expenditure and increased caloric intake may have contributed to the accumulation of fat reserves in our howler monkey population, which may explain the contrast between our data and other reports in the literature, but future comparative studies that monitor lean body mass across captive conditions are warranted.

Lastly, we found that lipase and albumin were not affected by age, sex, parasitism, and species. This finding may suggest that these proteins are more stable and less affected by intra- and inter-specific factors. Nevertheless, we recommend that the evaluation of these proteins is evaluated in conjunction with other examinations and clinical signs, as they could indicate specific conditions that were not present in our study.

In summary, our results showed that parasitism affected WBC and liver proteins and enzyme values. Controlling for species and age, sex affected platelets, and bilirubin concentration. Most parameters were affected by species or interaction between this factor and sex or parasites, as expected, indicating different physiological adaptations in two primate species characterized by distinct ecology and body size. Age affected RBC, WBC, platelet and MPV, and some liver protein and enzyme values, such as TP, globulin, A:G ratio, GGT, and ALP. We highlight the importance of characterizing parasite prevalence in primate populations, which is essential for monitoring their health, the efficacy of deworming procedures, and for management of their diet as well as hygienic measures to reduce pathogen transmission within the colon. Moreover, our comparative data suggest that hemogram and biochemistry parameters can provide valuable information associated with body mass, aging, and ecology, and will enable comparative studies with other platyrrhine primates to investigate interspecies differences in blood parameters as physiological adaptations and the role they played in human evolution.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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