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# Biochemical and hematological evaluations of black howler monkeys (*Alouatta pigra*) in highly degraded landscapes in Mexico

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## Abstract

**Background:** Loss and fragmentation of Neotropical primates' habitat can alter the health and individual adaptation. Physiological parameters reflect health status and individuals responses to the habitat conditions.

**Methods:** We captured six wild adult females and six adult males of *Alouatta pigra* to evaluate their physical condition (body mass, respiratory and heart rate, and rectal temperature), hematology and blood chemistry on fragmentation habitat condition at southeastern Mexico.

**Results:** The males weighed more than females, but the female' temperature, respiratory rate, and heart rate were higher. Low values of hemoglobin, hematocrit, and red blood cells suggest some degree of anemia in these howler monkeys. Low levels of the total protein, albumin, albumin/globulin Ratio, and low levels of blood urea nitrogen suggest that howler monkeys could have protein and carbohydrate deficiencies.

**Conclusions:** Howler monkeys living under the highly degraded conditions at Balancán, Tabasco, could be showing adaptability by modifying their physiology, hematology, and blood chemistry in response to the conditions of their fragmented landscape.

KEYWORDS
blood chemistry, clinical parameters, habitat fragmentation, hematology, physiological parameters

## 1 | INTRODUCTION

Some wildlife species can be used as sentinels of alterations that impact natural habitats.[1] Human activities and encroachment into natural habitats generally have negative impacts for wildlife conservation and can represent a risk to their health.[2] Hence, health status evaluation that determine hematological and physiological reference values of apparently healthy populations is a priority to develop conservation plans and to understand what factors could influence wildlife health.[3,4]

Primates are a good example of where the use of health evaluations to assess the level of infection of zoonotic pathogens[2] as a function of habitat quality[5] have aided in conservation planning. However, health evaluation need not be limited to epidemiological surveillance, but it can also consider animal's clinical and physiological parameters.[6] In fact, physiological parameters are a very important indicator of environmental disruption that can alter homeostasis.[4] One way to assess health status is to determine the hematological parameters of individuals.[2,7] Such assessments have been conducted in several species of wild primates in the Neotropics[4,5,8,9] and represent reference values for comparisons among intra and interspecies. However, caution is recommended in evaluating such assessments as environmental pressures can affect individuals or species differently.[7]

Loss and fragmentation of forests are the main threats to Neotropical primates; many species live in the canopy and thus have reduced ability to move across the landscape to obtain resources when the habitat is fragmented.[10-12] Fragmentation also alters the microclimate, affecting the quantity and nutritional quality of food.[11,13] Animals can respond to these changes through physiological adaptation which include changing physiology and blood parameters.[14,15]

In this study, we evaluated the health status of black howler monkeys (*Alouatta pigra*) in a highly degraded landscape of the State of Tabasco in Southeastern Mexico by measuring hematological and physiological parameters.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

Our study site is located in the 3626 km$^2$ system of plains and swamps of the Usumacinta River Basin in Tabasco, México (17°48′N, 91°32′W, 30 m above sea level; INEGI, 2014; Figure 1). The climate is warm and humid with a mean annual temperature of 27.6°C (minimum of 21.7°C, maximum 33.5°C), and a mean of 1818 mm of precipitation per year.[16] The municipality of Balancán is a highly perturbed landscape that has suffered extensive forest loss and fragmented habitat. During the 70′s through the 90′s the original flood plain forests experienced severe degradation, losing approximately 80% of their original cover, resulting in a patchwork of crops, cattle ranches, and degraded forest fragments.[12]

### 2.2 | Study subjects and immobilization

Following the protocols approved by the Animal Care Committee of Istituto de Ecología, A.C. and the approval of the Mexican agency Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; SGPA/DGVS/05938/08), between November 2008 and June 2009, we captured 12 adults (six females and six males)(Figure 1). One individual was selected from each troops belonging to different forest fragments. Each individual was considered representative of the troop due to semiclosed social structure of howler monkeys.[17]

Animals that appeared healthy were capture by a certified veterinarian by chemical restraint using tiletamine-zolazepam IM (Zoletil 50®, Virbac, France) at a dose of 15 mg/kg[7,8] or with ketamine hydrochloride IM (Anesket®, Pisa, Mexico) at a dose of 10-25 mg/kg.[18] The use of either anesthesia protocol depended on the height of the trees where monkeys were located; when the tree was very tall we used tiletamine-zolazepam to promote muscular relaxation. Intramuscular injection was achieved using plastic darts of 3 mL with needle of 1.2×38 mm, shot with a $CO_2$ rifle. A capture team caught the tranquilized monkey as it fell from the tree in a tarp. Immediately, after capture each monkey was screened for potential injuries caused by fall of which there were none. Every animal was weighed and measured and if necessary a supplementary dosage of ketamine hydrochloride (4 mg/kg) was administered. At the end of sample collection, animals were placed into a wire mesh cage until they fully recovered (1-2 hours) and then released near their group.

### 2.3 | Handling and sample collection

Once the animals were immobilized we placed them on a clean, dry, and smooth surface in a shaded area away from the group. During the first 10 minutes, each individual was subjected to a complete physical exam, that included oral, eyes, and ears examination and we registered any abnormalities. The weight of every animal was recorded. Rectal temperature, heart and lungs auscultation, and cardiac and respiratory rates were recorded at 10 minutes intervals throughout the procedure. The dart wound was located and treated with topical antibiotic ointment and ophthalmic ointment was applied to protect

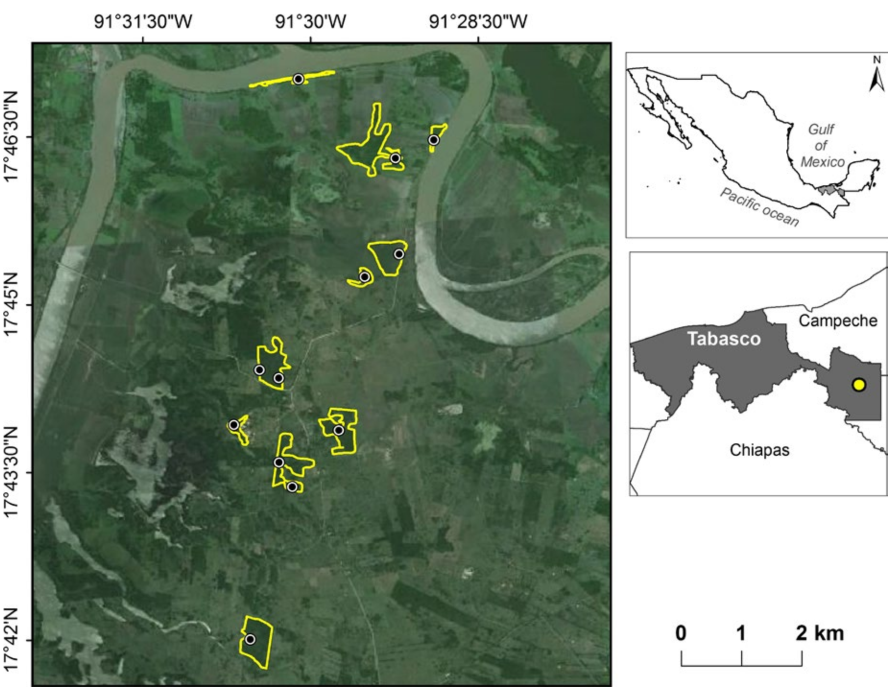


**FIGURE 1** Capture sites of black howler monkeys (*Alouatta pigra*) in the Balancán municipality, Mexico. The yellow polygons represent vegetation fragments remnants

corneas. Freeze gels packs and water-ethyl alcohol mix (50/50) were used to cool the animal if the rectal temperature rose to 39°C.

Blood samples (6-10 mL) were obtained from the femoral vein within 5 minutes after capture. Immediately after venipuncture, 2 mL of blood was stored in tubes containing sodium heparin (Vacutainer®; Benton Dickinson, Rutherford, NJ), another 2 mL was placed in ethylenediamine tetra-acetic acid (EDTA) tubes, and the remainder of the sample was placed in serum separator tubes. All samples were preserved at approximately 4°C in coolers with ice packs until they were processed, approximately 4 hours later.

Eighteen hematological parameters (Tables 1 and 2) were measured in an automated analyzer (Bayer Advia 60, SIEMENS; Erlangen, Germany), and 14 serum chemistries and enzymes were processed on a clinical chemical analyzer (Chem 7, Erba Mannheim; Mannheim, Germany) at a human laboratory in Balancán town. Not all individuals were sampled for all biochemical and hematological analyze, and such information was included in each table.

## 2.4 | Data analysis

The data of each physiological and hematological parameter were analyzed with a Kolmogorov-Smirnov test to determine its normality. Thereafter, we compared between sexes with a *t* test for independent variables or Mann-Whitney-Wilcoxon test depending on the normality of data.[19] We used STATISTICA version 7 software (StatSoft, Inc 2004, Tulsa OK, USA.) to run the tests, applying a significance level <0.05. Mean values are provided along with the corresponding standard deviation, median values, and minimum and maximum levels by sexes, and for all individuals. We used the physiological values reported to *A. pigra* by the International Species.[19] When comparing our blood panel and chemistry values to ISIS or those reported in the literature, statistical comparisons are not possible. For these comparisons, we only recorded which is higher and/or lower than the values from our study site in Balancán, and we show standard deviation for our values and explain them in tabular form.

## 3 | RESULTS

Body mass has significant different between sexes (t=5.96, *df*=8, *P*<.001). However, the sexes did not differ with respect to heart and respiratory rates and rectal temperature; nevertheless the females showed a tendency of higher values than males (HR, 140.0 hb/min female vs. 132.6 hb/min male; RR, 25.17 b/min vs. 21.8 b/min; RT, 38.63 vs. 38.5°C; Table 1). The red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), and the mean corpuscular hemoglobin concentration (MCHC) were higher in males compared with females (Table 2). The complete blood counts (hematology) did not differ between sexes; nonetheless, the white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), monocytes number (#MON), and granulocytes number (#GRA) were higher in females than males, by an average of 34% (1.51-fold). The blood chemistry showed sex differences in osmolarity (t=−2.61, *df*=9, *P*=.028),

**TABLE 1** Mean (±SD), median, minimum, and maximum values of body mass, heart and respiratory rates, and rectal temperature of captured *Alouatta pigra* from Balancán, Mexico for the entire sample of individuals and per gender

| | Entire Sample | | | | | | Females | | | | | | Males | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Mean | ±SD | Median | Min | Max | N | Mean | ±SD | Median | Min | Max | N | Mean | ±SD | Median | Min | Max | N | *P* |
| Weight (kg) | 7.20 | 1.62 | 7.50 | 5.00 | 9.00 | 10 | 5.50 | 0.58 | 5.50 | 5.00 | 6.00 | 4 | 8.33 | 0.82 | 8.50 | 7.00 | 9.00 | 6 | <0.001 |
| Heart Rate (heartbeats/min) | 134.18 | 25.70 | 12 | 101.00 | 180.00 | 11 | 140.40 | 28.79 | 144 | 106.00 | 180.00 | 5 | 132.6 | 25.2 | 128 | 101.0 | 170.0 | 6 | ns |
| Respiratory Rate (breaths/min) | 23.25 | 3.89 | 25.50 | 18.00 | 30.00 | 12 | 25.17 | 4.07 | 25 | 19.00 | 30.00 | 6 | 21.8 | 2.9 | 22 | 18.0 | 25.0 | 6 | ns |
| Rectal Temperature (°C) | 38.53 | 1.23 | 38.35 | 36.70 | 40.50 | 12 | 38.63 | 1.31 | 38.75 | 36.70 | 40.20 | 6 | 38.5 | 1.4 | 38.40 | 37.0 | 40.5 | 6 | ns |

Significance level: (ns) not significant.

**TABLE 2** Hematological parameters of captured male and female *Alouatta pigra* from Balancán, Mexico

| Parameters | Females N=3 | | | Males N=5 | | | *P* |
|---|---|---|---|---|---|---|---|
| | Mean±SD | Median | Min–max | Mean±SD | Median | Min–max | |
| Hb (g/dL) | 8.9±0.7 | 9.2 | 8.1- 9.4 | 10.6±1.6 | 10.7 | 9.0-12.3 | .39 |
| HCT (%) | 26.6±1.7 | 27.0 | 24.7-28 | 32.6±4.8 | 33.3 | 26.9-37.6 | .14 |
| RBC (x $10^6$/$mm^3$) | 3.1±0.2 | 3.2 | 2.9-3.3 | 4±0.6 | 3.8 | 3.3-4.7 | .11 |
| MCV (fL) | 85±1 | 85.0 | 84.0-86 | 81.6±4.5 | 82.0 | 75.0-87 | .26 |
| MCH (pg) | 28.5±0.4 | 28.7 | 28.0-28.8 | 26.7±1.7 | 27.6 | 23.9-28 | .13 |
| MCHC (g/dL) | 33.6±0.7 | 33.6 | 33.0-34.3 | 32.7±0.6 | 32.8 | 31.8-33.4 | .09 |
| PLT (x $10^3$/$mm^3$) | 299.7±191.2 | 201.0 | 178.0-520 | 214.8±95.2 | 213.0 | 126.0-354 | .42 |
| WBC (x $10^3$/$mm^3$) | 6.9±1 | 7.4 | 5.8-7.7 | 5.2±1.6 | 5.1 | 3.3-7.3 | .08 |
| MON (%) | 11.1±3.5 | 12.8 | 7.1-13.4 | 9.5±2.5 | 10.5 | 5.5-12 | .48 |
| LYM (%) | 48.4±14.9 | 44.6 | 35.8-64.9 | 53.8±5.9 | 55.5 | 45.2-59.8 | .48 |
| GRA (%) | 40.5±11.6 | 42.6 | 28.0-50.8 | 36.6±5.6 | 37.1 | 29.4-44.3 | .54 |
| PTC (%) | 0.37±0.20 | 0.28 | 0.24-0.60 | 0.27±0.10 | 0.25 | 0.17-0.40 | .350 |
| RDW (%) | 15.60±0.20 | 15.60 | 15.4-15.80 | 15.74±0.75 | 15.30 | 15.10-16.90 | .770 |
| MPV (µ$m^3$) | 12.90±1.15 | 13.30 | 11.6-13.80 | 12.86±1.35 | 12.90 | 11.40-14.90 | .968 |
| PID (%) | 14.93±1.27 | 14.20 | 14.20-16.40 | 15.24±1.00 | 15.10 | 14.20-16.90 | .571 |
| # LYM ($10^3$/$mm^3$) | 3.23±0.57 | 3.40 | 2.60-3.70 | 2.8±0.88 | 2.80 | 1.50-3.70 | .480 |
| # MON ($10^3$/$mm^3$) | 0.73±0.29 | 0.90 | 0.40-0.90 | 0.44±0.23 | 0.40 | 0.20-0.80 | .161 |
| # GRA ($10^3$/$mm^3$) | 3.00±1.15 | 3.40 | 1.70-3.90 | 1.98±0.56 | 2.10 | 1.40-2.80 | .135 |

Hb, hemoglobin; HCT, hematocrit; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets count; WBC, white blood cells; MON, monocytes percentage; LYM, lymphocytes percentage; GRA, granulocytes percentage; PTC, plasma thromboplastin component; RDW, red blood cell distribution width; MPV, mean platelets volume; PID, primary immunodeficiency disorders; #LYM, lymphocytes number; #MON, monocytes number and #GRA, granulocytes number.

total protein (t=−3.11, *df*=8, *P*=.014), globulin (t=−2.91, *df*=7, *P*=.023), and the common electrolytes (phosphorus, t=−2.47, *df*=9, *P*=.036; magnesium, t=−2.51, *df*=8, *P*=.037; chloride, t=−2.38, *df*=10, *P*=.038, Table 3).

## 4 | DISCUSSION

Physiological values represent important information to evaluate individual health status.[4,7,14,21,22] The physiological values commonly considered as reference are those reported by the International Species Information System.[20] However, the values are from captivity, the sample size is limited, and not all samples are from healthy animals. Wild animals' physiology can be considered as natural condition which it is used as reference to evaluate health status of captive animals. There are few records from wild populations of physiological and blood parameters of black howler monkeys; however, the studies of Abella-Medrano,[23] Rovirosa-Hernández et al.,[5] and Canales-Espinosa et al.[24] from the rainforest in Campeche and Tabasco, Mexico, provide data to compare with our results.

It is very important to assess body mass to calculate anesthesia dose and to know general body condition, as well as hydration level.[7] Balancán howlers males had 51.45% higher body mass than females, which is similar to that reported elsewhere (females 5.05 kg and males 7.05 kg[23]; females 5.05 kg and males 6.99 kg[5]; females 5.05 kg in Campeche and 5.88 kg in Tabasco, and males 6.99 kg in Campeche and 7.09 kg in Tabasco[24]). Black howler monkeys from Balancán were heavier than expected for animals in a highly degraded area, and they did not show signs associated with inappropriate diet or reduced food intake. It has been proposed that anatomy and physiology traits, behavioral ecology,[25,26] and its interactions with gut microbiota,[25,27] reflecting that the genus *Alouatta* can adapt well to structural changes in vegetation.[25-30]

Heart and respiratory rates vary depending on the methods and circumstances under which they are measured, changing during daytime due to environmental temperature and animal handling.[31,32] The rectal temperature registered in New World primates varies from 36 to 40°C[20], and the mean body temperature registered in ISIS database is lower than we observed (37.3±1.0°C, N=4 vs. 38.5±1.23°C, N=12, respectively), hence, our results are in the range reported for Neotropical primates. By the absence of the heart and respiratory rates data for *A. pigra*, we estimated the rates by allometric scaling with the metabolic constants for mammals,[33] which they consider the body mass; the heart and respiratory rate values estimated to black howlers monkeys are 147 heartbeats per minute and 32 breaths per minute (females: 157 hb/34 b; males: 142 hb/31 b). These calculated allometric rates are higher than the results we obtained; but our animals were under the effect of chemical immobilization.

**TABLE 3** Blood chemistry values of captured male and female *Alouatta pigra* from Balancán, Mexico

| | Females | | | | Males | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Mean±SD | Median | Min–max | N | Mean±SD | Median | Min–max | N | P |
| Glucose (mg/dL) | 84.80±28.15 | 91.0 | 37.00-112.00 | 5 | 99.20±20.91 | 97.0 | 75.00-130.00 | 5 | .208 |
| BUN (mg/dL) | 31.50±15.93 | 27.5 | 18.00-53.00 | 4 | 15.00 | 15.0 | | 1 | .138 |
| Total protein (g/dL) | 7.45±0.51 | 7.35 | 7.00-8.10 | 4 | 6.38±0.55 | 6.6 | 5.40-6.80 | 6 | .014 |
| Albumin (g/dL) | 2.68±0.29 | 2.7 | 2.20-3.00 | 5 | 2.48±0.22 | 2.4 | 2.30-2.80 | 5 | .257 |
| Globulin (g/dL) | 4.78±0.46 | 4.7 | 4.30-5.40 | 5 | 3.82±0.51 | 4.0 | 3.10-4.30 | 4 | .023 |
| Alb/Glob Ratio | 0.60±0.12 | 0.6 | 0.50-0.70 | 4 | 0.64±0.09 | 0.7 | 0.50-0.70 | 5 | .730 |
| Creatinine (mg/dL) | 0.83±0.19 | 0.75 | 0.70-1.10 | 5 | 1.10±0.39 | 1.0 | 0.70-1.60 | 4 | .238 |
| Cholesterol (mg/dL) | 81.00±12.47 | 82.0 | 66.00-95.00 | 5 | 105.67±34.13 | 103.5 | 66.00-167.00 | 6 | .162 |
| Triglycerides (mg/dL) | 117.83±86.92 | 94.0 | 38.00-278.00 | 6 | 63.67±22.99 | 70.0 | 35.00-87.00 | 5 | .240 |
| AP (U/L) | 349.17±199.06 | 337.5 | 90.00-669.00 | 6 | 236.17±62.64 | 242.5 | 124.00-297.00 | 6 | .214 |
| Calcium (mg/dL) | 8.48±3.44 | 9.75 | 1.50-10.40 | 6 | 7.88±3.13 | 8.85 | 1.60-9.90 | 6 | .240 |
| Phosphorous (mg/dL) | 3.92±1.16 | 4.3 | 1.90-4.80 | 5 | 2.53±0.69 | 2.25 | 1.80-3.40 | 6 | .036 |
| Magnesium (mg/dL) | 2.28±0.38 | 2.5 | 1.70-2.60 | 5 | 1.78±0.24 | 1.8 | 1.50-2.10 | 5 | .037 |
| Chloride (mmol $L^{-1}$) | 101.17±3.97 | 100.0 | 98.00-109.00 | 6 | 91.50±9.09 | 95.0 | 77.00-101.00 | 6 | .038 |
| Potassium (mmol $L^{-1}$) | 6.08±1.03 | 5.85 | 5.00-7.90 | 6 | 5.33±0.79 | 5.2 | 4.30-6.50 | 6 | .189 |
| Sodium (mmol $L^{-1}$) | 137.00±6.13 | 134.5 | 131.00-148.00 | 6 | 128.67±9.31 | 133.0 | 115.00-136.00 | 6 | .097 |
| Osmolarity (mOsm/L) | 275.83±10.26 | 273.5 | 265.00-294.00 | 6 | 255.80±15.12 | 265.0 | 236.00-268.00 | 5 | .028 |
| Bilirubin Conjugated (mg/dL) | 0.06±0.03 | 0.07 | 0.02-0.09 | 5 | 0.07±0.02 | 0.07 | 0.05-0.09 | 6 | ns |
| Bilirubin Unconjugated (mg/dL) | 0.15±0.05 | 0.13 | 0.10-0.23 | 5 | 0.13±0.07 | 0.15 | 0.02-0.19 | 6 | ns |
| Total Bilirubin (mg/dL) | 1.23±2.53 | 0.21 | 0.17-6.40 | 6 | 0.19±0.08 | 0.21 | 0.07-0.28 | 5 | ns |
| AST (SGOT) (IU/L) | 76.20±13.83 | 79.0 | 62.00-96.00 | 5 | 93.00±33.12 | 82.5 | 70.00-158.00 | 6 | ns |
| ALT (SGPT) (IU/L) | 27.60±3.71 | 27.0 | 23.00-33.00 | 5 | 27.50±4.14 | 27.5 | 22.00-33.00 | 6 | ns |
| LDH (IU/L) | 234.00±99.13 | 218.0 | 134.00-400.00 | 5 | 237.80±73.15 | 200.0 | 166.00-325.00 | 6 | ns |
| CK (IU/L) | 317.00±186.70 | 203.0 | 173.00-577.00 | 5 | 386.00±119.66 | 357.0 | 252.00-572.00 | 5 | ns |
| LDT (mg/dL) | 20.40 | | | 1 | 36.95±3.32 | 37.5 | 33.00-39.80 | 4 | |
| HDT (mg/dL) | 10.00 | | | 1 | 42.00±15.87 | 47.0 | 19.00-55.00 | 4 | |
| Amylase (U/L) | 376.00 | | | 1 | 317.75±93.64 | 344.5 | 183.00-399.00 | 4 | |
| Lipase (U/L) | 75.00 | | | 1 | 82.33±10.41 | 79.0 | 74.00-94.00 | 3 | |
| LDC (mg/dL) | 39.00 | | | 1 | 41.75±6.18 | 42.5 | 35.00-47.00 | 4 | |
| GGT (IU/L) | 51.00 | | | 1 | 36.25±26.09 | 41.5 | 3.00-59.00 | 3 | |

BUN, blood urea nitrogen; AP, alkaline phosphatase; AST, aspartate aminotransferase or SGOT; ALT, alanine aminotransferase or SGPT; LDH, lactate dehydrogenase; CK, creatine kinase; LDT, low density triglycerides; HDT, high density triglycerides; LDC, low density cholesterol; GGT, gamma-glutamyl transferase. Significance level: (ns) not significant.

Many of our hematological values are not consistent with reported values.[5,23,24] Hb, HCT, and RBC were slightly lower, but within the ranges reported to the specie.[5,23,24] We found no statistical differences between sexes for some parameters, such as HCT, even though males tend to have higher HCT values than females, these data agreed with Canales-Espinosa et al.[24], due to sex hormonal effects as androgen and erythropoietin have a stimulatory effect in male' bone marrow and erythropoietin production in the kidney, and estrogens have an inhibitory effect on the bone marrow in females.[14,34]

Compared with reported values,[5,20,23,24,40] Hb, HCT, and RBC values were slightly low suggesting that our sampled black howlers monkeys should be anemia signals,[22] probably produced by dietary deficiency or decreased nutrients absorption by dysbiosis.[35] However, WBC, MCV, and % monocytes are lower, and platelets (PLT) have higher concentrations than reported.[5,24] MCHC was higher than values reported, along to low HCT, could indicate a regenerative anemia,[22,23] but this could be evaluated by blood smears,[14,22] which was not possible in our study. ISIS values are higher in WBC, Hb, HCT, MCV, and #Lymphocytes, and lower in MCH, MCHC, and #MON were similar to our results; while other recorded parameters (RBC, MCH, MCHC, #MON) are within the ranges we observed. WBC was slightly higher in females than males, this probably is a characteristic of the genus *Alouatta*, which some authors suggest that leukocytosis could be produced by helminthic infection, capture procedure, and social factors.[24] Our hematological value differences indicate condition of non-physiological hemolysis. However, the hemolysis could be a result of sample handling, because the MCHC was slightly high, and the values of HCT and RBC were low,[14,22] and the samples had not turbidity to suggest lipemia in lab report.

In Balancán, total protein, albumin, Alb/Glob relation, and chloride are lower than reported;[5,24] the creatinine, triglycerides, calcium, phosphorus, magnesium covary with gender and have low concentrations. These low levels suggest that black howlers monkeys have poor protein intake; the blood urea nitrogen (BUN) levels are important to evaluate hepatic and renal function due protein metabolism[14] and our levels suggest dehydration or carbohydrate deficiency; in this case the carbohydrate deficiency may be the likely cause as the HCT is low, and in dehydration the HCT has high levels due to hemoconcentration.[14]

Compared to available data to black howler monkeys,[5,20,24] the howler monkeys from Balancán have lower values of alkaline phosphatase, glucose, creatinine, and calcium. The BUN, phosphorus, cholesterol, total protein, albumin, globulin, AST, total bilirubin, alkaline phosphatase, and creatine kinase are within the range of reported values.

During capture and handling, there are physiological changes produced by stress hormones that can be detected on short-to-medium term after capture.[35,36] For example, stress can alter the blood cell parameters.[14,38] The effect of post handling stress has been documented in primates and non-primates species.[32,38-40,43] Handling will cause the release of corticosterone and other stress hormones, and some blood parameters could be altered few hours later.[32,39] In many species, increasing stress hormones levels is associated with a decrease in the total number of white blood cells and an increase in the number of neutrophils,[39] HCT[14], and platelets.[38] Nonetheless, animal restraint usually has a small effect on lymphocyte numbers.[39] *A. pigra* has shown an increase of plasma cortisol within 20 minutes of chemical restraint.[41] Our results do not indicate a stress response because lymphocytes do not decrease and HCT is not increased relative to values reported.[5,24] Therefore, we recommend obtaining blood samples within three minutes of darting (see also[42]).

The anesthetics may affect physiological and blood parameters.[31] In the chemical restraint of *A. pigra*, platelet, lymphocyte and phosphorus concentration are reported to decrease and creatinine, cholesterol, triglyceride, and potassium increase.[43] We documented a minimum effect on the hematology; that is, the lowest platelet and lymphocyte numbers and the maximum creatinine, cholesterol, phosphorus, and potassium values were similar to the reported under ketamine effect after 10 minutes.[43]

Black howler monkey physiology and health could also be influenced by fragmentation and/or habitat lost.[15] In Mexican nonhuman primates (*Alouatta palliata*, *A. pigra*, and *Ateles geoffroyi*), the cortisol levels in individuals in fragmented habitats are chronically higher than in those found in preserved forests.[44-47] Stress hormones have an immediate effect[40] and could alter the hematological and biochemical parameters.[14,32,37-40]

The sampling was performed within the time when anesthetic had minimum effect on blood parameters; thus, we consider our values to represent normal values, to the extent possible for field situations, and allows comparisons to black howler monkeys at different localities and in different habitats. Our survey increases the comparative data base that will soon be robust enough to allow detailed quantitative comparisons among habitats, including anthropogenically modified forests.

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