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# Hematological and Biochemical Profile of *Callithrix kuhlii* (COIMBRA-FILHO, 1985) in Urban and Peri-Urban Areas of Southern Bahia, Brazil

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## ABSTRACT

Brazil harbors the greatest diversity of primates, and among them, the genus *Callithrix* is notable for its adaptation to urban environments. Despite being endemic to southern Bahia and northeastern Minas Gerais, the Wied's marmoset (*Callithrix kuhlii*) remains understudied, highlighting the need for baseline health data. This study aimed to characterize the hematological and biochemical profiles of *C. kuhlii* in urban and peri-urban areas within its distribution range. A total of 106 wild marmosets were captured, and blood samples were collected from the femoral plexus of the individuals. Hematological parameters showed no significant differences between sexes. However, sex-based variations were observed in serum biochemistry, with males showing higher triglycerides and urea levels, possibly influenced by dietary factors and testosterone. This pioneering study establishes essential baseline data on the hematology and biochemistry parameters of *C. kuhlii* in the wild, supporting future research on its ecology and conservation within the Atlantic Forest.

## 1 | Introduction

Non-human primates (NHPs) inhabit highly diverse ecosystems, particularly in tropical regions, and are found across four continents: Africa, the Americas, Asia, and Europe [1]. Brazil harbors the greatest diversity of known NHPs, with 139 taxa—including species and subspecies (IUCN, 2023)—distributed across various biomes [2]. The Atlantic Forest alone is home to 23 species [3]. In the state of Bahia (BA), 14 NHP species have been recorded [4], two of which are endemic: *C. kuhlii*—even though the species can also be found in a small area in Minas Gerais (MG)—and *Lentopithecus chrysomelas* [5, 6]. Approximately 95% of the *C. kuhlii's* distribution falls within Bahia [7].

The genus *Callithrix* comprises six species commonly known as marmosets: *C. geoffroyi* (É. Geoffroy in Humboldt, 1812), *C. penicillata* (É. Geoffroy, 1812), *C. aurita* (É. Geoffroy in Humboldt, 1812), *C. flaviceps* (Thomas, 1903), *C. jacchus* (Linnaeus, 1758), and *C. kuhlii* (Coimbra-Filho, 1985). These species exhibit a remarkable ability to adapt to urban environments. Coupled with the ongoing fragmentation of the Atlantic Forest, this adaptability has led to increasingly synanthropic behavior [8].

Knowledge about *C. kuhlii* (Figure 1) remains limited, partly due to ongoing debates regarding its taxonomic classification [9–11]. These primates have been considered either a distinct species, hybrids of *C. geoffroyi* and *C. penicillata*, or a variation

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**FIGURE 1** | Specimen of *Callithrix kuhlii*. *Source:* Pedro Japiassu, Bahia, Brasil, 2023.

of *C. penicillata* [7]. Geographically, *C. kuhlii* is restricted to the region between the Jequitinhonha river (MG) to the south, the Rio de Contas (BA) to the north, and the elevated terrains of the Conquista plateau (BA) to the west, spanning parts of Bahia and Minas Gerais states [7]. The ongoing habitat fragmentation and increasing urbanization have forced these primates to adapt to human-modified landscapes. As they adjust to diverse environments, their presence and health status can serve as valuable bioindicators, reflecting ecological changes such as deforestation and urban expansion [12]. They also harbor various pathogens, including viruses, bacteria, and parasites [13]. Although their adaptation to human-modified environments increases disease transmission risks, it also allows them to function as sentinels for emerging infectious diseases [14–16].

The fragmentation of ecosystems due to rapid urbanization, deforestation, and agricultural expansion poses a significant threat to *C. kuhlii*, leading to its classification as a vulnerable species by the International Union for Conservation of Nature [2]. Habitat fragmentation can isolate populations, limiting migration opportunities and increasing susceptibility to climatic variations, natural disasters, demographic stochasticity, and inbreeding, ultimately elevating the risk of extinction [17, 18].

Assessing the health of NHPs often requires capturing and examining individuals, yet studies on the physiological profiles of Neotropical primates remain scarce due to logistical challenges [19]. While physical examinations provide valuable insights, many diseases manifest with nonspecific signs, and wild animals often conceal symptoms [14]. Thus, laboratory analyses, particularly hematological and biochemical tests, are essential for accurate diagnosis and health monitoring, offering a cost-effective approach to establishing baseline parameters [20, 21].

Despite its ecological importance, the health parameters of *C. kuhlii* remain poorly understood [22], particularly in free-living populations. This study addresses this gap by providing baseline hematological and biochemical data on wild individuals from southern Bahia, Brazil.

## 2 | Materials and Methods

### 2.1 | Humane Care Guidelines

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. This study complied with the Principles for the Ethical Treatment of Non-Human Primates established by the American Society of Primatologists (ASP) and followed the Code of Best Practices for Field Primatology to minimize stress on the animals. Additionally, all procedures adhered to the legal requirements of Brazil. The project was approved under license No. 023/20 by the Ethics Committee for Animal Use of the Universidade Estadual de Santa Cruz (CEUA/UESC, No. 023/20), the Chico Mendes Institute for Biodiversity Conservation (ICMBio—SISBIO No. 75734-1), and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN, No. AF40BCA).

### 2.2 | Research Location

The study was conducted in pre-defined regions of southern Bahia, randomly selected based on the estimated distribution of *C. kuhlii* [7]. In this study, the species' occurrence area was delineated using the Minimum Convex Polygon technique, with data points plotted using ArcGIS 9.0 software. The resulting polygon covered an area of 23 300 km [2], incorporating records of *C. kuhlii* occurrence. The southernmost point was located in Salto da Divisa, Minas Gerais; the westernmost in Bandeira, Minas Gerais, and the northernmost in Ipiaú, Bahia, with the Atlantic Ocean serving as the eastern boundary. To define sampling points and generate maps, QGIS 3.22.10 was used, ensuring a random spatial distribution. Study areas were selected based on the species geographical range, with a preference for sites farther from the distributional boundary to minimize the potential for hybridizations with other callitrichids (Figure 2). To characterize the habitats where captures took place, land use in the study region was analysed using Google Earth Engine (2013) [23] and MapBiomas (2023) [24]. Additionally, the normalized difference vegetation index (NDVI) was applied to assess vegetation cover at the capture points, providing detailed environmental data [25] (Online resource: Figure S1; Table S1).

### 2.3 | Experimental Design and Capture

To determine the sample size, the simple random sampling method was applied [26]. The estimated population size of 10 000 individuals was obtained from the IUCN database (2023) [2]. Using a 90% confidence level and a 10% margin of error, the required minimum sample size was calculated to be 68 animals.

The animals were captured between January and August 2023 using Tomahawk automatic traps (20 cm x 20 cm x 60 cm). The

traps were secured in selected sites, such as trees, with No. 22 galvanized wire [27]. Trap placement was guided by observations of animal movement and feeding behavior, and fruits such as bananas and mangoes were used as bait (Figure 3). Additionally, the playback technique was employed, which involves playing *Callithrix* vocalizations to attract individuals, as these primates are territorial and respond to conspecific calls [28]. For this, a JBL speaker was positioned near the traps, broadcasting *Callithrix* sp. vocalizations to enhance capture efficiency.

## 2.4 | Animal Handling and Pre-Anesthesia Period

Upon capture, the animals were kept inside the traps at the capture sites for 30 to 45 min to attract other group members. During this period, they were continuously monitored to avoid sun exposure and to detect any signs of discomfort. Subsequently, researchers approached and secured traps with wire to prevent escapes [27]. Meanwhile, a temporary field laboratory for sample collection was set up at least 20 m away to minimize stress.

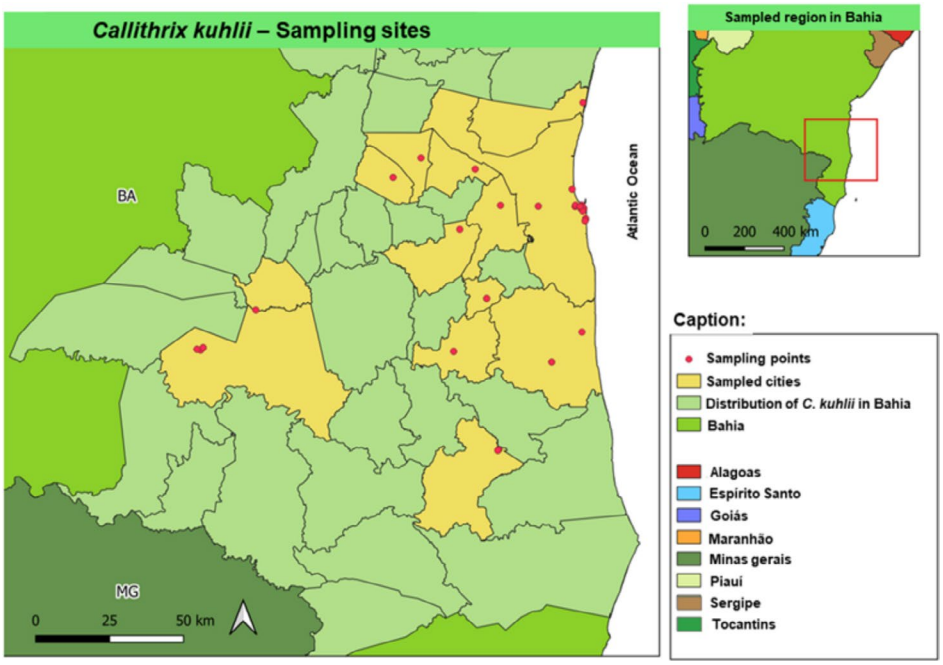


**FIGURE 2** | Map indicating the visited municipalities and collection sites of *C. kuhlii* in Bahia, 2023.


**FIGURE 3** | Tomahawk traps arranged in locations for capturing *C. kuhlii*. (a) Traps distributed on tree branches where the animals were seen feeding, with the sound box (blue) used in playback technique visible. (b) Traps arranged on construction structures where the animals had been observed moving. (c) Traps placed on existing structures in the home of a citizen who feeds the animals. Bahia, 2023 (CEUA/UESC, No. 023/20; ICMBio—SISBIO No. 75734-1; SISGEN, No. AF40BCA).

After the initial waiting period, the animals were carefully transported to a shaded and quiet area. To further reduce stress and minimize the risk of injury, the traps were covered with cloth [27]. Restricting an animal's visual field has been shown to lower distress levels during handling and restraint in various wild species [29]. While inside the covered traps, the animals were continuously monitored through visual observation, in which a researcher would lift the cloth periodically to check for signs of distress—such as increased aggression, excessive activity, rapid or labored breathing, and abnormal posture—as well as audible signs, including vocalizations [28]. The animals remained in the traps for a total of at least 2 h from the moment of capture. This duration allowed for a fasting period, reducing the risk of aspiration during anesthesia induction and minimizing potential alterations in hematological and biochemical parameters, which could affect the interpretation of the results [27, 30].

### 2.5 | Anesthesia and Clinical Evaluation

Next, the researchers, wearing personal protective equipment (PPE), including KN95 masks, procedural gloves, and surgical gowns—physically restrained the primates using leather gloves (Figure 4). Once restrained, anesthesia induction and maintenance were performed with isoflurane (1 mL/mL) at a minimum alveolar concentration (MAC) of 2%, administered via the VetBag—Anesthetic Backpack through an inhalation mask. The backpack operates as an open system connected to an oxygen cylinder and utilizes a universal vaporizer [27]. Isoflurane is one of the most commonly used inhalation anesthetics in primates, ensuring prolonged unconsciousness, supplemental oxygen supply, and improved muscle relaxation [31]. Additionally, recovery from inhalation anesthesia is faster than from dissociative agents, as it is not accumulative in the body and does not rely on hepatic metabolism for elimination [32].

Following anesthesia induction, the animals underwent a comprehensive clinical evaluation, including morphometry measurements (head-body length, head circumference, tail length, and weight), ectoparasite screening, assessment of dental condition, integumentary integrity, oral cavity, anus, and genitals, as well as inspection for fractures or amputations. Throughout the procedure, vital parameters were monitored every 5 min, including: body temperature (digital thermometer; Winner.med), heart rate (stethoscope; Littmann Classic III), respiratory rate (by observing diaphragmatic movement), and reflex tests (palpebral, interdigital, and anal). The callitrichids were classified as juveniles or adults based on weight, following the criteria described in the literature [33]. After age and sex determination, sample collection was initiated. Morphometry data were analyzed separately for adults, juveniles, males, and females (Table S2). After clinical evaluation and induction of anesthesia, biological sample collections began.

### 2.6 | Blood Collection

After asepsis with alcohol and cotton, approximately 3 mL of blood was collected via venipuncture of the femoral plexus, ensuring that the volume did not exceed 1% of the animal's body weight [30] (Figure 5). Syringes (3 mL) with 0.45 × 13 mm needles were used for collection, and hemostasis was achieved by applying dry cotton to the puncture site.

Blood samples were then transferred into two types of tubes: EDTA-containing tubes (0.5 mL; Labor Import) (0.5 mL) for hematological analysis, and tubes without anticoagulant but with clot activator gel (2.5 mL; Labor Import) for serum biochemistry analysis. Samples were stored in isothermal boxes with ice, maintaining a temperature of 2 to 8°C until processing.


**FIGURE 4** | Procedures conducted after the individuals' capture. (a) Physical restraint with suede gloves and chemical restraint with inhaled Isoflurane using the VetBag—Anesthetic Backpack. (b) Vital parameter measurement through a multiparameter monitor and maintenance of anesthesia. (c) Measurement of heart rate using a stethoscope and collection of *C. kuhlii* samples. (d) Example of clinical evaluation conducted during the procedure, oral inspection performed on *C. kuhlii* (CEUA/UESC, No. 023/20; ICMBio—SISBIO No. 75734-1; SISGEN, No. AF40BCA).


**FIGURE 5** | Procedures performed on *C. kuhlii*. (a) Blood collection through venipuncture in the femoral plexus. (b) Tubes used for blood allocation, tube with purple cap containing EDTA (0.5 mL) and tube with yellow cap without anticoagulants and with clot activator gel (2.5 mL). (c) Collection of biometric data from sampled individuals. (d) Inter-scapular microchipping for animal marking. Bahia, 2023 (CEUA/UESC, No. 023/20; ICMBio—SISBIO No. 75734-1; SISGEN, No. AF40BCA).

## 2.7 | Exclusion Criteria and Animal Release

Pregnant animals, offspring, or those weighing less than 200 g [33] were excluded from the study due to their higher susceptibility to stress, which could result in mortality or abortion. After fully recovering from anesthesia—confirmed by behavioral assessment—all animals were released at their exact capture location. Releases occurred before 4:00 PM to accommodate their diurnal activity patterns. Notably, the entire procedure lasted an average of 15 min, which was crucial in minimizing stress on the animals, and after blood collection, the samples were promptly processed for detailed hematological and biochemical analyses, as outlined in the methods section below.

## 2.8 | Sample Processing

### 2.8.1 | Hematological Analysis

Hematological profiles were assessed within 48 h post-collection using manual and visual techniques [34]. The following parameters were measured: Erythrocyte parameters: erythrocyte count (10 [6]/μL), hemoglobin (Hb) (g/dL), hematocrit (Ht) (%), mean corpuscular volume (MCV) (fl/L), mean corpuscular hemoglobin concentration (MCHC) (g/dL), mean corpuscular hemoglobin (MCH) (pg). Leukocyte parameters: total leukocyte count (cells/μL), and differential leukocyte count, including eosinophils (%), lymphocytes (%), monocytes (%), basophils (%), and neutrophils (%). Plasma protein concentration (PPT, g/dL), determined by refractometry (HT211, e-Labshop Brasil). Blood smears were prepared from whole blood for differential leukocyte and platelet counts.

For erythrocyte count per microliter of blood and leukogram analysis, a Neubauer chamber was used with dilution performed via an automatic pipette (Kacil), following the methodology described by Thrall (2015). Hematocrit (Ht) was determined using a microhematocrit tube filled approximately ¾ full through capillary action. The reading was performed using a hematocrit reader card, with results expressed as a percentage [35]. PPT was measured by refractometry. After breaking the capillary tube above the buffy coat, the plasma was transferred to the refractometer and analyzed according to its g/dL scale [35].

To determine hemoglobin (Hb) concentration, hematimetric indices were calculated, including mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH), following the methodology described in the literature [36]. Additionally, mean corpuscular volume (MCV), an index used to assess red blood cell size, was calculated by dividing hematocrit (Ht) by erythrocyte count [36].

For differential white blood cell counts and platelet counts, blood smears were prepared according to the method previously described [35]. Smears were fixed and stained using rapid panoptic stain (Laborclin), following the manufacturer's instructions. Once stained, slides were examined under a microscope. Leukocyte identification was based on morphological characteristics [37, 38]. For platelet counts, a drop of immersion oil was applied, and the counting was performed

under 1000x magnification, following standard protocols in the literature [35].

Hematological values were analyzed between groups (males vs. females and juveniles vs. adults), as these factors may influence hematological parameters due to variations in hormonal regulation, immune function, and body mass [39, 40].

#### 2.8.2 | Serum Biochemistry Analysis

The first step in the serum biochemistry analysis involved separating the serum from the clot. This was achieved by centrifuging the samples for 5 min at 5000 rpm. The serum was then aspirated using a pipette and transferred into 2 mL polypropylene tubes (Eppendorf) [35].

The following biochemical parameters were analyzed: cholesterol (mg/dL), triglycerides (TGL) (mg/dL), albumin (g/dL), urea (mg/dL), creatinine (mg/dL), alkaline phosphatase (FA) (IU/L), creatine kinase (CK) (IU/L), total serum proteins (PT) (g/dL), aspartate aminotransferase (AST) (IU/L), and alanine aminotransferase (ALT) (IU/L). The assays were performed using reagents from commercial biochemical kits (Labtest), with readings taken on a semi-automatic biochemical analyzer (Bioplus 2000). Globulins (g/dL) were calculated using the formula: globulins = total proteins (PT)—albumin. The only parameter measured using EDTA-treated blood was hemoglobin (g/dL). All assays were carried out in accordance with the manufacturer's dilution recommendations (Labtest). Additionally, glucose (g/dL) was measured using a portable glucose meter (On Call Plus II) during blood collection.

Age and sex can significantly influence serum biochemical parameters in mammals, and these factors should be considered when interpreting laboratory results [35]. Therefore, serum biochemistry data were compared between groups (males vs. females).

### 2.9 | Statistical Data Analysis

Hematological and biochemical data were summarized using descriptive statistics, including mean, standard deviation, range (maximum and minimum values), 95% confidence interval, and standard error. The normality of the data was assessed using the Shapiro–Wilk test. The appropriate statistical test (Mann–Whitney U or t-test) was then applied to evaluate significant differences between the sexes (males and females). Statistical analyses were performed using the software Tinn-R 8.02.02.01 (scripts are available in the Supporting Information S1), with a significance level of 5% ($p \leq 0.05$) [41]. To assess differences in vegetation status (NDVI) among capture points, a Kruskal–Wallis test was conducted, with 30 groups randomly divided into three subsets.

## 3 | Results

Between January and July 2023, 106 *C. kuhlii* individuals were captured across 13 municipalities in Bahia. The distribution of Wied's marmosets was as follows: Ilhéus (38), Una (22), Mascote (8), Arataca (3), São José das Vitórias (4), Itape (1), Itajuípe (2), Uruçuca (1), Itabuna (2), Itapetinga (15), Itororó (4), Coaraci (2), and Almadina (4) (Figure 2). An exact number of individuals at each sampling site is provided in the Supporting Information S1, with an average of three individuals per site across all sampling locations (Table S1). The study areas exhibited similar land cover, predominantly consisting of forest mosaics and agricultural areas, as determined through Google Earth Engine [23]

**TABLE 1** | Hematological parameters of *Callithrix kuhlii* in southern Bahia, 2023.

| | Measure | *N* | Mean | Min-Max | SD (±) | SE (±) | CI (95%) |
|---|---|---|---|---|---|---|---|
| MCHC | g/dL | 66 | 32.43 | 27.14–36.67 | 2.27 | 0.28 | 31.88–32.99 |
| MCH | pg | 66 | 22.63 | 14.18–32.3 | 3.83 | 0.47 | 21.69–23.57 |
| Hematimetry | $10^6/mm^3$ | 106 | 6.36 | 3.58–9.4 | 1.12 | 0.11 | 6.14–6.58 |
| Hematocrit | % | 105 | 45.49 | 34.4–57 | 5 | 0.49 | 44.52–46.46 |
| Hemoglobin | g/dL | 70 | 14.75 | 11.3–18.5 | 1.71 | 0.2 | 14.35–15.16 |
| Total Leukocytes | $mm^3$ | 106 | 7176 | 1450–15 350 | 3353 | 325 | 6531–7822 |
| Platelets | $10^3/mm^3$ | 104 | 462 788 | 198 000–749 000 | 124 271 | 12 185 | 438 620–486 956 |
| Proteins | g/dL | 97 | 6.66 | 3.9–8.9 | 1.23 | 0.12 | 6.41–6.91 |
| MCV | fL | 97 | 70.26 | 50–100 | 11.04 | 1.12 | 68.04–72.49 |
| Basophil | $mm^3$ | 95 | 52 | 0–219 | 60 | 6 | 40–65 |
| Eosinophil | $mm^3$ | 104 | 0 | 0 | 0 | 0 | 0 |
| Lymphocyte | $mm^3$ | 92 | 3159 | 194–6265 | 1445 | 150 | 2859–3458 |
| Monocyte | $mm^3$ | 99 | 63 | 0–297 | 73 | 7 | 48–77 |
| Neutrophil | $mm^3$ | 94 | 2858 | 361–7095 | 1478 | 150 | 2555–3161 |

Abbreviations: CI, confidence interval; Max, maximum; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Min, minimum; N, number of individuals; SD, standard deviation; SE, standard error.

**TABLE 2** | Hematological values of male and female *Callithrix kuhlii* in southern Bahia, 2023.

| | | Male | | | | | | Female | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Measure** | ***N*** | **Mean** | **Min-Max** | **DP (±)** | **EP (±)** | **IC (95%)** | ***N*** | **Mean** | **Min-Max** | **SD (±)** | **SE (±)** | **CI (95%)** | ***p*** |
| Hematimetry | $10^6/mm^3$ | 68 | 6.39 | 4.2–9.4 | 1.12 | 0.14 | 6.12–6.66 | 38 | 6.3 | 3.58–9 | 1.15 | 0.19 | 5.93–6.68 | 0.7028 |
| Hematocrit | % | 67 | 45.33 | 34.40–57 | 5.16 | 0.63 | 44.07–46.59 | 38 | 45.77 | 37–56 | 4.75 | 0.77 | 44.21–47.34 | 0.6641 |
| Hemoglobin | g/dL | 45 | 14.78 | 11.3–18.5 | 1.71 | 0.25 | 14.27–15.3 | 27 | 14.71 | 9.5–20.2 | 2.23 | 0.43 | 13.83–15.59 | 0.8758 |
| Lymphocyte | $mm^3$ | 59 | 3189.78 | 1291.5–6204 | 1236.56 | 160.99 | 2867.53–3512.03 | 34 | 3245.41 | 194–7904 | 1935.72 | 331.97 | 2570–3920.82 | 0.6975 |
| MCH | pg | 43 | 23.05 | 16.13–32.3 | 4.08 | 0.62 | 21.79–24.3 | 23 | 21.85 | 14.17–30 | 3.25 | 0.68 | 20.45–23.26 | 0.9582 |
| MCHC | g/dL | 41 | 32.84 | 28.81–36.67 | 1.88 | 0.29 | 32.24–33.43 | 24 | 31.84 | 25.69–36.4 | 2.83 | 0.58 | 30.65–33.03 | 0.4672 |
| MCV | fL | 63 | 69.83 | 51.16–95.94 | 11.01 | 1.39 | 67.06–72.6 | 35 | 72.09 | 50–102.85 | 12.57 | 2.12 | 67.77–76.4 | 0.3012 |
| Platelets | $10^3/mm^3$ | 61 | 442098.4 | 239000–663000 | 95236.85 | 12193.83 | 417707.1–466489.7 | 38 | 447026.3 | 99000–749000 | 149830.1 | 24305.65 | 397778.4–496274.2 | 0.7643 |
| Proteins | g/dL | 61 | 6.68 | 4–8.9 | 1.16 | 0.15 | 6.39–6.98 | 38 | 6.44 | 3–8.8 | 1.54 | 0.25 | 5.93–6.94 | 0.9654 |
| Total Leukocytes | $mm^3$ | 66 | 6752.42 | 1900–13800 | 2844.26 | 350.1 | 6053.22–7451.63 | 38 | 7517.11 | 1450–15350 | 3766.97 | 611.08 | 6278.93–8755.28 | 0.6073 |
| Neutrophil | $mm^3$ | 63 | 2742.74 | 361–6566 | 1414.91 | 178.26 | 2386.4–3099.08 | 33 | 3366.83 | 487.5–7812 | 1894.08 | 329.72 | 2695.22–4038.45 | 0.0991 |
| Lymphocyte | $mm^3$ | 59 | 3189.78 | 1291.5–6204 | 1236.56 | 160.99 | 2867.53–3512.03 | 34 | 3245.41 | 194–7904 | 1935.72 | 331.97 | 2570–3920.82 | 0.6975 |
| Basophil | $mm^3$ | 61 | 49.95 | 0–219.2 | 56.95 | 7.29 | 35.37–64.54 | 35 | 63.83 | 0–268 | 73.9 | 12.49 | 38.44–89.21 | 0.8880 |
| Eosinophil | $mm^3$ | 66 | — | — | — | — | — | 36 | — | — | — | — | — | — |
| Monocyte | $mm^3$ | 64 | 57.17 | 0–234 | 63.89 | 7.99 | 41.21–73.13 | 34 | 67.69 | 0–291 | 30 | 13.72 | 39.78–95.61 | 0.6071 |

Abbreviations: CI, confidence interval; Max, maximum; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Min, minimum; N, number of individuals; SD, standard deviation; SE, standard error.

and MapBiomas [24]. Additionally, NDVI was calculated to further assess vegetation patterns, showing no significant differences among the locations ($p < 0.0889$) (Figure S1; Table S1).

All animals were clinically healthy, with no signs of fever, hemorrhage, apathy, or amputations upon physical examination. No significant differences in morphological features between sexes were observed (Table S3). However, due to the limited number of juveniles captured, a robust statistical analysis based on age groups could not be performed.

### 3.1 | Hemogram

The hematological and WBC data for 106 individuals, including both sexes and all age groups, are presented in Table 1. The complete data can also be found in the Table S4.

No significant differences were observed in hematological and WBC parameters between females and males ($p > 0.05$) (Table 2).

In addition to the hematological analysis, biochemical values were assessed, with the species-specific results presented in Table 3. The complete dataset is available in the Table S5.

Regarding sex differences in serum biochemistry, males showed significantly higher levels of TGL and urea, while ALT showed a marginally significant difference ($p \leq 0.05$) (Table 4).

## 4 | Discussion

Our study determined the hematological and serum biochemical profile of *C. kuhlii* from urban and peri-urban areas of Southern Bahia, using a larger sample size than any previous research on wild individuals of the genus *Callithrix* [18, 42–46]. We likely captured individuals from 30 different groups; however, we could not determine the exact number of individuals per group, as population sizes may vary [47] (Table S1). Although the study areas exhibited disturbed forests and areas with anthropogenic influence (e.g., forest mosaics and agroforestry lands), consistent with previous studies with other primate species in the same distribuition [48], the animals captured in these environments may exhibit hematological and biochemical differences, potentially related to the altered habitats (Figure S1).

The hematological data described in this study are consistent with those reported for other species [18, 43, 45] and with the study of *C. kuhlii* in 1993 [46]. Comparing leukograms across species may reveal differences due to genetic variability and the challenges faced by animals in different habitats [49]. However, it is important to emphasize the difference in sample sizes, with statistical analysis being necessary to yield more reliable results that accurately represent the population [50]. Additionally, it is crucial to consider the differences between free-living and captive animals, as the latter may exhibit significant differences in feeding behavior and stress levels. Confined animals often cannot exhibit natural behaviors, potentially leading to behavioral or welfare impairments [51]. Our findings regarding the hematological profiles of *C. kuhlii* were largely consistent with previous studies [18, 46], though slight differences may be attributed to habitat conditions or sample handling. For instance, the higher levels of TGL observed in males align with those of the species *Callithrix penicillata* [44], suggesting a potential dietary influence.

Furthermore, the lack of significant differences in hematological parameters between males and females supports previous studies in *Callithrix* species [18, 46, 52], suggesting that this species may not exhibit significant sexual dimorphism. However, when comparing serum biochemical data, we observed that males had higher levels of TGL and urea, potentially linked to dietary habits and hormonal differences [53, 54].

Biochemical indicators directly reflect the nutritional status of animals [55, 56], as well as modifications in renal and hepatic function

**TABLE 3** | Biochemical data for free-living *Callithrix kuhlii* in southern Bahia, 2023.

| | Measure | *N* | Mean | Min-Max | SD (±) | SE (±) | CI (95%) |
|---|---|---|---|---|---|---|---|
| Albumin | g/dL | 70 | 4.25 | 0.6–8.38 | 1.68 | 0.2 | 3.85–4.65 |
| ALT | UI/L | 57 | 7.11 | 5–15 | 3.13 | 0.41 | 6.29–7.93 |
| AST | UI/L | 62 | 143.06 | 73–240 | 42 | 5.33 | 132.4–153.72 |
| CK | UI/L | 62 | 856 | 121–2526 | 604.42 | 76.76 | 702.51–1009.49 |
| Cholesterol | mg/dL | 56 | 221.82 | 23–488 | 122.53 | 16.37 | 189.01–254.63 |
| Creatinine | mg/dL | 38 | 0.75 | 0.75–0.75 | 0 | 0 | 0.75–0.75 |
| Alkaline Phosphatase | UI/L | 62 | 164.39 | 8–373 | 72.54 | 9.21 | 145.97–182.81 |
| Glucose | mg/dL | 79 | 263.97 | 117–471 | 87.47 | 9.84 | 244.38–283.56 |
| Globulin | g/dL | 67 | 2.69 | 0.04–6.74 | 1.47 | 0.18 | 2.33–3.05 |
| PT | g/dL | 64 | 6.58 | 5.1–8.6 | 0.7 | 0.09 | 6.4–6.76 |
| Triglycerides | mg/dL | 55 | 180.98 | 1–556 | 174.55 | 23.54 | 133.79–228.17 |
| Urea | mg/dL | 52 | 23.46 | 3–48 | 11.13 | 1.54 | 20.37–26.55 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; PT, total proteins.

**TABLE 4** | Serum biochemistry comparison between male and female *Callithrix kuhlii* in southern Bahia, 2023.

| | | Males | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Measure** | ***N*** | **Mean** | **Min-Max** | **SD (±)** | **SE (±)** | **CI (95%)** | ***N*** | **Mean** | **Min-Max** | **SD (±)** | **SE (±)** | **CI (95%)** | ***p*** |
| Albumin | g/dL | 42 | 4.34 | 1.5–8.38 | 1.65 | 0.25 | 3.84–4.84 | 26 | 3.8 | 0.6–7.26 | 1.36 | 0.27 | 3.24–4.36 | 0.6164 |
| ALT | UI/L | 36 | 6.53 | 5–10 | 2.34 | 0.39 | 5.74–7.32 | 23 | 9.39 | 5–26 | 5.83 | 1.22 | 6.86–11.92 | 0.0596** |
| AST | UI/L | 33 | 137.06 | 73–225 | 34.68 | 6.04 | 124.76–149.36 | 29 | 151.52 | 79–282 | 52.34 | 9.72 | 131.61–171.43 | 0.6853 |
| CK | UI/L | 38 | 790.42 | 121–2574 | 633.78 | 102.81 | 582.11–998.73 | 25 | 1024.4 | 291–2526 | 629.21 | 125.84 | 764.68–1284.12 | 0.1063 |
| Cholesterol | mg/dL | 33 | 207.52 | 23–384 | 110.68 | 19.27 | 168.27–246.77 | 23 | 242.35 | 37–488 | 137.72 | 28.72 | 182.79–301.91 | 0.4732 |
| Creatinine | mg/dL | 21 | 0.75 | 0.75 | 0 | 0 | 0.75 | 17 | 0.75 | 0.75 | 0 | 0 | 0.75 | — |
| Alkaline Phosphatase | UI/L | 34 | 154.79 | 8–315 | 65.14 | 11.17 | 132.06–177.52 | 28 | 176.04 | 33–373 | 80.28 | 15.17 | 144.91–207.17 | 0.6585 |
| Glucose | mg/dL | 47 | 257.87 | 117–456 | 79.37 | 11.58 | 234.56–281.18 | 29 | 252.59 | 138–438 | 78.96 | 14.66 | 222.56–282.62 | 0.3624 |
| Globulin | g/dL | 39 | 2.6 | 0.21–6 | 1.47 | 0.24 | 2.11–3.09 | 25 | 2.62 | 0.33–4.34 | 0.94 | 0.19 | 2.23–3.01 | 0.7664 |
| PT | g/dL | 38 | 6.64 | 5.1–8.6 | 0.69 | 0.11 | 6.42–6.86 | 26 | 6.5 | 5.2–8.3 | 0.72 | 0.14 | 6.21–6.79 | 0.4793 |
| Triglycerides | mg/dL | 36 | 253.58 | 10–790 | 219.03 | 36.51 | 179.46–327.7 | 18 | 66.78 | 1–264 | 72.88 | 17.18 | 30.53–103.03 | 0.0049* |
| Urea | mg/dL | 26 | 26.62 | 5–48 | 10.51 | 2.06 | 22.38–30.86 | 25 | 21 | 3–45 | 10.66 | 2.13 | 16.6–25.4 | 0.0454* |

Abbreviations: ALT, alanine aminotransferase; CK, creatine kinase; PT, total proteins; ST, aspartate aminotransferase.
*$p \leq 0.05$.
**Marginally significant.

or discrepancies in water intake [35]. Regarding serum biochemistry, a study reported data on wild *Callithrix* species [57], including glucose (171.1 ± 63.4 mg/dL), cholesterol (139.3 ± 34.8 mg/dL), and urea (21.8 ± 6.3 mg/dL), with the first two parameters differing from those found in the present study (263.97 ± 87 mg/dL; 221.82 ± 122.53 mg/dL, respectively). When compared to other species (wild *C. jacchus* [58]; *C. penicillata* in captivity [59]), fluctuations in values were observed, with the parameters in this study being more similar to those reported in *C. penicillata* in captivity [59] for TGL, AST, CK, and creatinine. The urea values are similar to those reported in wild *C. jacchus* [58] and for the genus also in the wild [57], and albumin levels were consistent only with those described by the first author [58]. The other parameters (glucose, globulin, PT, cholesterol, ALT) did not show similar results, and FA was not reported in the studies compared. Variations in blood chemical composition among mammals can be attributed to several factors, such as differences in sample size, handling, anesthesia protocols, as well as metabolic differences between species (e.g., male vs. female, age group), habitat, and diet [60].

Regarding sex differences, higher values of TGL and urea were observed in males in this study. Although studies with *C. geoffroyi* [18] in urban areas did not find significant differences, they did report higher TGL levels in males. The increase in TGL for males is consistent with findings in *C. penicillata* [44] in captivity. Elevated TGL levels may indicate the consumption of lipid- and carbohydrate-rich foods, reduced lipase activity, or a genetic defect affecting this enzyme [61]. Seasonal variations and rainfall levels can also influence the diet of callitrichids, with animals in wetter climates prioritizing fruits and those in drier areas feeding more on sap and insects [62]. Additionally, hormonal differences, such as higher testosterone levels in males, can contribute to increased TGL levels [63]. Our study, conducted between February and July, coincided with a period of fruit scarcity, which may also explain our findings, as the capture areas were urban and periurban, where animals may have relied more on carbohydrate-rich foods or human-provided food sources.

Regarding urea, which was also higher in males of *C. kuhlii* in our study, studies with *C. geoffroyi* [18] and with *C. penicillata* [44] reported the opposite, with females showing higher values, although the differences were not statistically significant. Urea is a metabolite influenced by nutrition, particularly high protein intake, and thus, dietary disparities between sexes could affect serum urea levels [56]. The protein content in an insect-based diet may have contributed to the observed urea levels in males in our study, or hormonal differences, with testosterone potentially influencing protein metabolism in males [64].

A limitation of our study is the variation in sample sizes between sexes and age groups, which may have influenced the statistical power of some analyses. Standardizing the sample distribution, particularly balancing the number of males, females, juveniles, and adults, would enhance the robustness of future studies. However, achieving this standardization in wild populations remains a challenge. Despite this limitation, our study provides a hematological and biochemical reference profile for *C. kuhlii* in its natural habitat, an essential step for understanding the species' health. Given the increasing human presence in these environments, further research on different demographic groups is necessary. Additionally, as a sentinel and bioindicator species, *C. kuhlii* plays a crucial role in health and disease monitoring in primates.

In conclusion, our findings provide crucial baseline data on the hematological and biochemical profiles of *C. kuhlii*, emphasizing the need for further studies on the species in its natural habitat. These results are vital not only for understanding the health of *C. kuhlii* but also for informing conservation strategies and One Health initiatives, given the species' role as a sentinel for primate health in anthropogenically influenced environments.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The datasets generated during and/or analyzed during the current study are available in the FigShare repository, https://doi.org/10.6084/m9.figshare.28608344.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.