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Age, sex, year season, and handling system modify the leukocytal parameters from captive *Caiman latirostris* and *Caiman yacare* (Crocodylia: Alligatoridae)

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Abstract

Mussart, N.B.; Barboza, N.N.; Fioranelli, S.A.; Koza, G.A.; Prado, W.S.; Coppo, J.A.: ***Age, sex, year season, and handling system modify the leukocytal parameters from captive Caiman latirostris and Caiman yacare (Crocodylia: Alligatoridae).*** *Rev. vet. 17: 1, 3–10, 2006.* The purpose of the study was to establish the reference interval for blood leukocytes from captive northeastern Argentine caimans, as well as to detect physiological variations related to species, sex, age, year season, and handling system. Samples from 223 healthy sub–adults, both sexes specimens of *Caiman latirostris* (n = 109) and *Caiman yacare* (n = 114), were obtained. White blood cells concentration (13.7±2.5 G/l) and proportion of heterophils (17.5±3.4%), lymphocytes (75.0±6.8%), monocytes (5.3±1.1%), eosinophils (2.2±0.4%), and basophils (0.3±0.05%) were determined by reptilian specific techniques. For each type of cell, largest diameters were, respectively: 14.49±1.54; 8.70±1.01; 12.74±1.85; 12.55±2.15 and 10.77±1.02 um. Heterophils rate was significantly higher in *C. yacare* than in *C. latirostris* ($p < 0.05$); lymphocytes rate showed an inverse relationship between species. Lymphocytes proportion was significantly higher in females than in males. The growth progress was reflected in slight heterophils decrease and lymphocytes increase. Total leukocytes concentration and lymphocytes percentage were significantly lower in autumn and winter. Decreases of heterophils (not significant) and monocytes (significant) were registered in summer and spring. Greater leukocytal differences attributable to handling systems (housing, feeding) used in a hatchery and a zoo, were not verified. Obtained data are applicable for the diagnosis and prevention of diseases in these species.

Key words: *Caiman latirostris*, *Caiman yacare*, leukocyte concentration, differential count, physiological variations.

Resumen

Mussart, N.B.; Barboza, N.N.; Fioranelli, S.A.; Koza, G.A.; Prado, W.S.; Coppo, J.A.: ***Edad, sexo, estación del año y sistema de manejo modifican los parámetros leucocitarios durante el cautiverio de Caiman latirostris y Caiman yacare (Crocodylia: Alligatoridae)****. Rev. vet. 17: 1, 3–10, 2006.* El propósito del estudio fue establecer el intervalo de referencia para el leucograma de caimanes autóctonos del nordeste argentino mantenidos en cautiverio, así como detectar variaciones fisiológicas atribuibles a especie, sexo, edad, estación del año y sistema de manejo. Se emplearon 223 ejemplares sanos, sub–adultos de ambos sexos, de las especies *Caiman latirostris* (n = 109) y *Caiman yacare* (n = 114). Mediante técnicas específicas para reptiles se determinó la concentración de glóbulos blancos (13,7±2,5 G/l) y se establecieron las proporciones de heterófilos (17,5±3,4%), linfocitos (75,0±6,8%), monocitos (5,3±1,1%), eosinófilos (2,2±0,4%) y basófilos (0,3±0,05%). En el mismo orden, los diámetros mayores de cada leucocito fueron: 14,49±1,54; 8,70±1,01; 12,74±1,85; 12,55±2,15 y 10,77±1,02 um. La tasa de heterófilos fue significativamente más alta en *C. yacare* que en *C. latirostris* ($p < 0,05$), en tanto que para los linfocitos ocurrió a la inversa. La proporción de linfocitos fue significativamente mayor en hembras que en machos. El avance del crecimiento se reflejó en ligeras disminuciones de heterófilos y aumentos de linfocitos. La concentración de leucocitos totales y el porcentaje de linfocitos fueron significativamente más bajos en otoño e invierno. En verano y primavera se registraron disminuciones de heterófilos (no significativas) y monocitos (significativas). No se verificaron grandes diferencias leucocitarias

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atribuibles a los sistemas de manejo (alojamiento, alimentación) aplicados en un criadero y un zoológico. Los datos obtenidos son aplicables al diagnóstico y prevención de las enfermedades de estos reptiles.

Palabras clave: *Caiman latirostris*, *Caiman yacare*, concentración de leucocitos, recuento diferencial, variaciones fisiológicas.

INTRODUCTION

Caiman latirostris (Daudin 1801) and *Caiman yacare* (Daudin 1802) are autochthonous species that inhabit fresh water of our country. Argentine caiman population was diminishing until the decade of 1980, when severe controls against stealthy hunt were imposed. Nowaday, these measures and the introduction of rearing programs as *ranching* system, allowed an increase of its populational density [30].

The *ranching* system consists on the gathering of eggs from wild nests, and an ulterior captive rearing, with restitution of juvenile caimans to the same environment, in a similar number to the proportion of specimens that would have survived under natural conditions, in safeguard of the ecological balance [19]. The age determination of reptiles is impossible if date of birth is ignored [5]. Therefore, caimans are divided in 4 important biological categories, according to their total longitude: I (0.23–0.40 m: younger than one year), II (0.41–1.30 m: sub–adults), III (1.31–1.70 m: adult reproducers, males and females), and IV (> 1.70 m: mature males) [30].

Adults caimans are unselective carnivores that can consume either live animals or carrion; in hatcheries they are maintained on balanced food [19]. Environmental temperature is important in feeding behavior and the digestive process. Most crocodilians cease feeding when the temperature goes below 25ºC [13], and their metabolism markedly will decrease due to fast [25]. This situation can cause variations on blood components [2, 29].

Hematological studies in wild life or captive crocodiles are being carried out for scientific, educational or commercial reasons; they are being applied to conservation or reproduction projects, such as to skin and meat exploitation [18]. Owing to the fact that every fragment of the crocodile can be made valuable, and also the endurance of crocodile to various diseases, quite a few farmers have turn their interest into crocodile farming [11].

Successful in animal disease control and prevention depend greatly on a precise and rapid diagnosis. This is a key to improve the crocodile production industry. Among several parameters, total white blood cells concentration and leukocyte differential count are definitely necessary in assisting clinicians to proximate the status of the animal body [11].

Development in diagnosis of the crocodile diseases has not gone far enough to a satisfy level. Additional information concerning the morphological and physiological characteristics of crocodilian blood leukocytes are needed to make a successful differential diagnosis and disease monitoring [11]. Apart from the concentration of white blood cells, differences in form and size of nucleus, cytoplasm, and staining characteristics of cell constituent of each leukocytes can be an integral indicator to identify the specific leukocyte type [2].

Knowledge of leukocytes and their physiological variations are incomplete and controversial in autochthonous caimans. Lack of uniformity on leukocyte denominations, such as a confusion caused by the different staining affinity from each one, are frequently verified in publications. In crocodile, heterophil is used in place of neutrophil as its granules have strong affinity to eosin; they become acidophilic and some time very much resemble the eosinophil. Moreover, there are confusion between "azurophil" (dropped out term) and monocyte or lymphocyte [9]. Authors report that azurophil has the appearance of mammal neutrophil mixed with monocyte [7].

Nowadays exists a reasonable consent to standardize the reptile leukocyte nomenclature to that of mammals; neutrophils are the exception because they are denominated heterophils. Under normal conditions, crocodile circulating blood has heterophils, lymphocytes, monocytes, eosinophils, and basophils [2, 15, 16, 17, 18, 22].

The primary function of heterophils is phagocytosis; they participate in inflammatory responses associated with microbial infections, parasitic diseases, and nonspecific inflammation (stress). Reptilian lymphocytes have a heterogeneity that goes beyond the level of T and B cell diversity. Lymphocytes found in peripheral blood originate from the thymus, bone marrow, spleen and other lymphopoietic crocodrile tissues. The B lymphocytes produce several types of immunoglobulins and the T lymphocytes moderate the immune responses. Lymphocyte concentration varies during wound healing, inflammation, and parasitic and viral infections [2]. Crocodile monocytes, heterophils and, in lesser extent eosinophils, possess phagocytic activity [14].

Monocytes participate in inflammatory responses and play an active role in granuloma and giant cell formation, especially in responses to bacterial and parasitic infections. They also participate in antigen–specific immunoglobulin interactions. The eosinophil concentration is influenced by parasitic and nonspecific stimuli; some reptilian eosinophils phagocytize immune complexes, indicating their participation in immune responses. Basophils have surface immunoglobulins and releasing histamine on degranulation. The number of circulating basophils may increase with certain blood parasites and virus infections [2].

The objective of this study was to obtain the reference interval for blood leukocytes from autochthonous captive healthy sub–adults *C. latirostris* and *C. yacare* specimens, as well as to establish eventual differences related to species, sex, age, year season, and handling system.

MATERIAL AND METHODS

Experimental subjects. During 2 years, 223 sub-adults [30] caimans, 50% of each sex, clinically healthy, from *C. latirostris* (n = 109) and *C. yacare* (n = 114) species, were studied. Some of them (n = 29) were maintained at the Corrientes City´s Zoo, in small ponds, without roof, with running water, which was constantly renovated; they were fed once per week on chicken viscera and fish. Remaining animals (n = 194) were housed at the "El Cachapé" farm (Chaco province), in roofed tanks with underground water, which was renewed dayly and stayed at 27 ± 3ºC (heated by gas), fed three times per week on meat flour supplemented with vitamins and minerals; sporadically they received bovine viscera [19]. To evaluate growth, reptiles were divided in 3 development stages, considering liveweight and corporal lenght.

Take of samples. Morphometric studies and blood extractions were performed 4 times a year, in each season, during morning hours (8–9 am), after 12 hours fast, without anesthetics neither tranquilizers. Liveweight was obtained using a hanging roman scales and corporal dimensions were evaluated with a metallic tape measure. Blood was collected by venipuncture of supravertebral occipital venous sinus [10], while reptile was manually held and its jaw remained tied. To minimize the stress [6, 12] such maneuvers were skillfully and quickly carried out by specialized personnel. Blood was treated with EDTA anticoagulant [2] and was maintained cooled at 5ºC until its analysis, which was made before 3 hours of extraction.

Laboratory techniques. White blood cells concentration was determined by microscopic recount in a Neubauer hemocytometer, using specific–reptilian methods and reagents [2, 8, 18]. Leukocytary formula was carried out by differential recount on blood smears stained by May Grünwald–Giemsa. Leukocyte dimensions were measured by an micrometric ocular [2, 16], in microscope Zeiss ST–25; photomicrographies were obtained by a Leica MC–80 camera.

Statistical analysis. Distributive normality was verified by Wilk–Shapiro test (WS). Parametric statistics included measures from central tendency (arithmetic mean, $\bar{x}$) and dispersion (standard deviation, SD). Fiduciary probability was evaluated by confidence intervals (CI±95%). Homogeneity of the variance was verified by Bartlett test. Analysis of variance (ANOVA) was made by one way linear model. Mean comparisons were carried out by Tukey test. Calculations were made with the aid of a statistical software (*Statistix* 1996). For all inferences a 5% significance was specified, below which the equality null hypothesis was rejected.

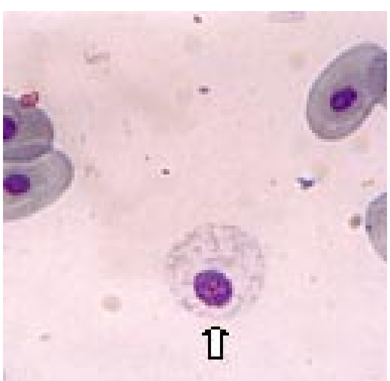

Figure 1. Heterophil of *Caiman sp.* May Grünwald–Giemsa, 1000x.

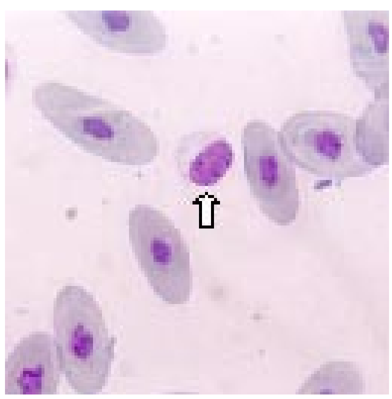

Figure 2. Lymphocyte of *Caiman sp.* May Grünwald–Giemsa, 1000x.

RESULTS AND DISCUSSION

Five types of leukocytes were considered, in agreement to recommendations from several authors [2, 15–18, 22]; "neutrophil" was referred as heterophil, and "azurophils" were assimilated to monocytes. Two types of heterophils, active and inactive, are mentioned by some investigators [20]. On the other hand, six types of white blood cells are considered by others, because azurophils and monocytes are described

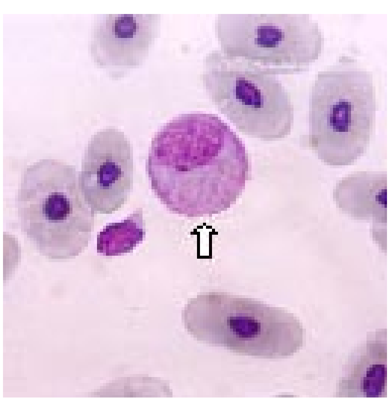

Figure 3. Monocyte of *Caiman sp.* May Grünwald–Giemsa, 1000x.

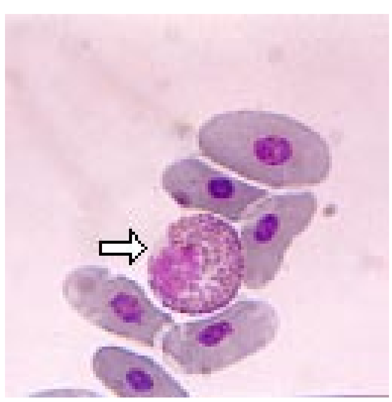

Figure 4. Eosinophil of *Caiman sp.* May Grünwald–Giemsa, 1000x.

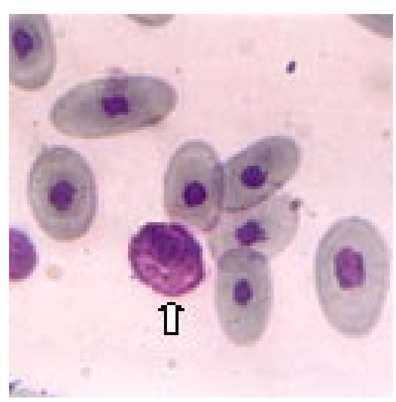

Figure 5. Basophil of *Caiman sp.* May Grünwald–Giemsa, 1000x.

as different cells [11, 27]. The lack of multiple nuclear segmentations in crocodile leukocytes, was corroborated [18]. Heterophils were the largest cells and lymphocytes were the smallest ones on reptile blood smears.

Heterophils from *C. latirostris* and *C. yacare* (Figure 1) showed rounded contour, cytoplasm with varied coloration polymorphic granules, and elliptic, eccentric nucleus. Reptilian heterophils are recognized by their eosinophilic, fusiform cytoplasmic granules; they are large cells with a round to oval, eccentric nucleous, and fusiform bright orange cytoplasmic granules (Wright stain) [2]. Heterophil from captive adult *Crocodylus siamensis* has oval, eccentric nucleus, with mostly spiculate granules and a few tear drop–shaped granules (Wright–Giemsa stain) [11]. Wild adults *Paleosuchus trigonatus* showed polymorphic contour heterophils, with pink or violet cytoplasm, and outlying violet nucleus in their "inactive type", such as cytoplasmic blued coma–shaped granules in their "active type" (Wright stain) [20]. Heterophis from *C. yacare* are spherical cells, with eccentric or outlying nucleus, and cytoplasmic polymorphic granules (fusiform, oval, spherical or stick form), with irregular surfaces [16].

Lymphocytes from caimans of present study (Figure 2) were small cells, with lightly basophil cytoplasm, and irregular contour nucleus occupying great part of the cytoplasm. Reptilian lymphocytes are, in general, mononuclear round cells that tend to mold or fold around adjacent cells in the blood films. The basophilic cytoplasm is typically scant, and may has azurophilic granules [2]. Lymphocyte from *C. siamensis* has eccentric kidney shaped nucleus, with scant bluish grey granules [11]. In *P. trigonatus* lymphocytes are round, with rosy cytoplasm, rounded and blued nucleus that occupies almost the whole cytoplasm [20]. Polymorphic lymphocytes with irregular contour nucleus, scarce cytoplasm, and azurophils granules, were recognized in *C. yacare* [16].

In the current study, monocytes (Figure 3) appeared as big cells, with basophilic cytoplasm and spherical eccentric nucleus, in coincidence to morphological characteristics described for *C. yacare* "azurophils": spherical cells, with basophilic eccentric nucleus, and basophilic cytoplasm with azurophils granules [16]. In general, reptile monocytes have a single nucleus that differs in shape but is typically ovoid and indented. The cytoplasm is finely granular, and is often vacuolated. Monocytes with an azurophilic appearance to the cytoplasma are often referred to as azurophils [2]. In *P. trigonatus* they are polymorphic cells, with rosy or transparent cytoplasm, round or polymorphic red nucleus [20]. Monocytes from *C. siamensis* has eccentric kidney shaped nucleus, and abundant and foamy, bluish grey cytoplasma granules, but "azurophils" cells reveal centrally located round, oval or kidney shaped nucleus, with small amount of azurophilic granules [11].

Studied eosinophils (Figure 4) were round cells, with abundant eosinophilic round cytoplasmic granules, and low–cut nucleus (bilobed in some cases). In coincidence, other works revealed that eosinophils from *C. yacare* had eccentric, rounded, oval, or lobed nucleus, with prominent nucleolus; their cytoplasm was occupied by big, spherical, compact, eosinophilic, and uniform size granules [17]. Spherical eosinophils, with eccentric or outlying lenticular nucleus, and cytoplasm with spherical or oval voluminous granules, were described in the same species [16]. Eosinophil from *C. siamensis* has round or oval eccentric nucleus, with round, rod, and few tear drop–shaped and spiculate cytoplasm granules [11]. *P. trigonatus* eosinophils are polimorphic cells, with rosy cytoplasm, and red polimorphic nucleus [20]. It is described that reptilian eosinophils are large, round cells with spherical, eosinophilic, cytoplasmic granules. The centrally positioned nucleus is variable in shape, ranging from slightly elongated to lobed [2].

Basophils from Argentine northeastern caimans (Figure 5) were small cells, with abundant basophil granules that hindered the visualization of their central nucleus. Their characteristics were not different from those described for other reptiles. Reptilian basophils are typically small spherical cells filled with basophilic metachromatic granules; the nonlobed nucleus is slightly eccentric and often obscured by the cytoplasmic granules [2]. *C. siamensis* has basophils with centrally located round nucleus, and its cytoplasm is full of round various sizes of granules [11]. In *P. trigonatus* they are round cells, with blue cytoplasm and blued round nucleus that almost occupies the whole cytoplasm [20]. Basophils from *C. yacare* was described as spherical cells of small size, with central spherical nucleus, and cytoplasm with scarce azurophil granules [16].

Morphological leukocyte alterations, as stain affinity change, left shift, cytoplasm vacuolation, and presence of toxic granules, are important diagnosis guides. Structural changes of circulating lymphocytes, such as lymphocytes kept into the thymus and spleen, were verified in *Alligator mississippiensis* specimens contamined by agricultural pesticides [21].

Table 1 exhibit the results of white blood cells micrometry from grouped species, since ANOVA did not reveal significant differences ($p < 0.05$) of leukocytal size among species, sexes, ages, year seasons, and handling systems. WS coefficients reveal an approximately normal distribution. Data dispersion (SD) did not exceed the limits that are recommended by parametric statistics. Confidence intervals were adjusted around arithmetic means, but individual ranges were something wide, in coincidence with descriptions made by other authors [2].

The heterophil diameter obtained in present study is similar to those reported for *Cayman crocodylus*: 11–19 um [22], and reptiles in general: 10–23 um [2]. The size was smaller in *C. siamensis*: 9–10 um [11], and bigger in *P. trigonatus*: 12–25 um (inactive type) and 19–23 um (active type) [20]. The size of lymphocyte from *C. latirostris* and *C. yacare* was bigger than that reported for *C. siamensis*: 6–7 um [11], and smaller than that published for *P. trigonatus*: 9–11 um [20]. On the other hand, lymphocyte

Table 1. Size obtained for each leukocyte (um).

parameter	$\bar{x}$ ± SD	WS	CI ± 95%	range
heterophils	14.49 ± 1.54	0.9342	14.24–14.73	11.27–17.42
lymphocytes	8.70 ± 1.01	0.9218	8.54–8.87	7.17–10.25
monocytes	12.74 ± 1.85	0.9592	12.45–13.03	7.17–17.42
eosinophils	12.55 ± 2.15	0.9605	12.20–12.90	8.20–16.40
basophils	10.77 ± 1.02	0.9133	10.54–11.01	9.22–12.30

Size: longer diameter, $\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk–Shapiro distributive normality test (critical value: 0.947, $\alpha = 0.05$), CI ± 95%: confidence interval.

Table 2. Leukocyte counts obtained in total studied population (n = 223).

parameter	$\bar{x}$ ± SD	WS	CI ± 95%	range
total leukocytes (G/l)	13.7 ± 2.5	0.966	13.1–14.3	6.7–24.2
heterophils (%)	17.5 ± 3.4	0.971	16.6–18.4	8–29
lymphocytes (%)	75.0 ± 6.8	0.968	73.9–76.1	62–87
monocytes (%)	5.3 ± 1.1	0.932	4.9–5.7	2–12
eosinophils (%)	2.2 ± 0.4	0.768	1.9–2.4	1–9
basophils (%)	0.3 ± 0.05	0.599	0.2–0.4	0–2

$\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk–Shapiro distributive normality test (critical value: 0.947, $\alpha = 0.05$), CI ± 95%: confidence interval.

size was similar to those described for *C. crocodylus*: 6.5–9 um [22], and for reptiles in general: 5–10 um [2].

Dimension obtained here for monocyte diameter coincides with the reported for *C. crocodylus*: 11.5–14.5 um [22], but is smaller than that encountered in *P. trigonatus*: 21–25 um [20]. Contrarily, monocytes from *C. siamensis* were smaller: 10–16 um (azurophils: 8–10 um) [11]. Other authors affirm that monocytes are generally the largest leukocytes found in the peripheral blood of reptiles (8–20 um) [2]. The size of Eosinophil of current study were similar to those found in *C. crocodylus*: 11.5–15 um [22] and *P. trigonatus*: 13–15 um [20], but they were bigger than that reported for *C. siamensis*: 8–9 um [11]. Diameter here obtained for basophils is similar to those found in *C. siamensis*: 9–11 um [11] and *P. trigonatus*: 8–10 um [20], although it was lower than that described for *C. crocodylus*: 12–15 um [22]. It is affirmed that reptilian basophils have a diameter from 7–20 um [23].

Table 2 shows the results of total and differential white blood cells counts for grouped both species. Dispersion of data was scarce, but individual ranges were very wide. In agreement, it is affirmed that reptile hematological values widely fluctuate into the same species [2, 29]. Eventual variations due to postprandial effect, circadian rhythm, and drugs influence, were excluded from the experimental design, because samples were taken during fast, in uniform morning hours, without medications [3]. In crocodiles, the lack of fast causes blood changes and interferes with analytical procedures [15].

Mean white blood cells value obtained here was similar to those found in *A. mississippiensis*: 12.26±5.6 G/l [25] and *Crocodylus niloticus*: 15±4 G/l [31]. The range reported for *Crocodylus porosus*: 6.4 to 25.7 G/l [15] also coincides with our results. In other cases, means were lightly lower (*C. crocodylus*: 10.4±1.6 G/l) [22] or markedly lower (*A. mississippiensis*: 6.4±2.9 G/l) [14].

Leukocytary formula revealed a net prevalence of lymphocytes over the other cellular types in our caimans, in disagreement to statements that the numbers of heterophils in peripheral blood of normal reptiles can represent up to 40% of the leukocyte differential count [2]. Heterophils rate from *C. latirostris* and *C. yacare* coincides to that reported for *C. porosus*: 15 to 23% [15] but is lower than those found in *C. niloticus*: 51.5% [31], *C. crocodylus*: 51.3±11% [22] and *A. mississippiensis*: 35% [25] and 54.7% [14].

We coincide with the affirmation that lymphocytes tend to be the most prevalent leukocyte in peripheral blood (80%) of some reptilian species [2]. Values obtained here coincide to that registered on *C. porosus*: 69–85% [15]. Other investigations report lower values in *C. niloticus*: 20.6% [31], *C. crocodylus*: 20±4% [22] and *A. misissippiensis*: 32% [25] and 23.9% [14].

Monocytes proportion registered here is similar to that published for *C. niloticus*: 5.1% [31], but is higher than those found in *A. mississippiensis*: 0–7% [14] and *C. porosus*: 0–4% [15]. In other assays monocytes rate was higher (*A. mississippiensis*: 12% [25] and *C. crocodylus*: 7.84±5.16% [22]). It is described that monocytes generally occur in low numbers in the peripheral blood of most reptiles, and range between 0 and 10% of the leukocyte differential count [4].

Rate of eosinophils obtained in our caimans is agree to those published on *C. niloticus*: 2.1% [31] and *C. porosus*: 0–2% [15], but is lower than those reported on *C. crocodylus*: 15.30±11.58% [22] and *A. mississippiensis*: 10.4% [14] and 12% [25]. Our basophils average coincides to that found in *C. porosus*: 0–1% [15]. Remaining reported values are higher: 4.8% in *C. crocodylus* [22], 20.6% in *C. niloticus* [31], and 8% [25] y 12.7% [14] in *A. mississippiensis*. In present study, basophils registered the lowest concentrations among blood smear cells. It is affirmed that basophils can represent 0 to 40% of the normal leukocyte differential count of reptiles [2].

Table 3 shows the leukogram values discriminated by species. No significant differences were detected for total white blood cells count, but heterophils proportion was significantly higher in *C. yacare* and lymphocytes percentage was greater in *C. latirostris*. Similar values of total leukocytes were found in Argentine northeastern captive juvenile specimens (liveweight: 540–780 g, total longitude: 72 cm) of *C. latirostris*: 12.51±3.21 G/l (n = 12) and *C. yacare*: 17.20±3.76 G/l (n = 5), although individual ranges were wider: 9–18 and 12–19 G/l respectively [29].

Table 3. Leukocyte values obtained on each species.

parameter	*C. latirostris* (n = 109)		*C. yacare* (n = 114)	
	$\bar{x}$ ± SD	CI ± 95%	$\bar{x}$ ± SD	CI ± 95%
total leukocytes (G/l)	13.5 ± 2.2	12.7–14.3	13.8 ± 2.6	13.1–14.6
heterophils (%)	16.3 ± 3.1 [a]	14.9–17.8	18.4 ± 3.5 [b]	17.2–19.6
lymphocytes (%)	77.2 ± 6.4 [a]	75.5–78.7	73.5 ± 6.6 [b]	71.8–74.8
monocytes (%)	5.2 ± 0.9	4.7–5.7	5.4 ± 1.2	4.8–5.9
eosinophils (%)	2.3 ± 0.4	1.9–2.5	2.1 ± 0.3	1.8–2.4
basophils (%)	0.5 ± 0.06	0.4–0.7	0.2 ± 0.04	0.1–0.3

$\bar{x}$: arithmetic mean, SD: standard deviation, CI ± 95%: confidence interval. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 4. Variations according to sex, liveweight, and total lenght in both species ($\bar{x}$).

parameter	sex		liveweight (kg)			total lenght (cm)		
	male	female	< 3.5	3.5–5.0	> 5	< 100	100–110	> 110
total leukocytes (G/l)	14.3	13.8	14.2	13.1	13.9	13.6	13.4	14.2
heterophils (%)	18.2	17.5	18.6	17.5	16.4	17.9	17.2	17.0
lymphocytes (%)	72.0 [a]	75.4 [b]	74.3	75.4	76.2	73.5	75.0	75.9
monocytes (%)	5.7	5.3	5.1	5.5	5.0	5.3	5.2	5.6
eosinophils (%)	2.4	2.1	2.1	2.8	2.2	2.4	1.9	2.1
basophils (%)	0.3	0.2	0.2	0.4	0.3	0.2	0.3	0.3

$\bar{x}$: arithmetic mean. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 5. Variations according to year season and handling systems ($\bar{x}$).

parameter	year season				handling	
	spring	summer	autumn	winter	farm	zoo
total leukocytes (G/l)	15.1 [a]	14.9 [a]	12.3 [b]	11.4 [b]	13.1	14.5
heterophils (%)	17.3	16.3	18.9	18.7	17.6	16.4
lymphocytes (%)	76.2 [a]	76.3 [a]	72.5 [b]	72.2 [b]	75.4	74.8
monocytes (%)	4.9 [a]	4.2 [a]	6.3 [b]	7.1 [b]	5.1	5.4
eosinophils (%)	1.9	2.2	2.4	2.2	2.0	2.4
basophils (%)	0.5	0.2	0.4	0.3	0.3	0.2

$\bar{x}$: arithmetic mean. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

In the same zone, total leukocytes of captive juvenile both sex specimens from *C. latirostris* and *C. yacare* (n = 20) were something higher (22.3±4.3 and 16±8.5 G/l respectively) [27]. Also lightly higher were the white blood cells registered in *C. latirostris* from southern Brazil (15–16 G/l) on samples obtained by cardiac puncture [8]. Markedly higher were total leukocytes found in *C. latirostris* by electronic count (136 G/l) [24]; this technique is not reliable in reptiles due to interferences caused by nuclei of red blood cells [2].

C. latirostris and *C. yacare* rates of heterophils (3±2 vs 4±3%), lymphocytes (66±6 vs 61±8%), monocytes (6±3 vs 5±0.6%), eosinophils (18±5 vs 21±4%), basophils (1±0.2 vs 0%) and azurophils (5±2 vs 8±2%), were respectively determined on northeastern Argentine specimens [27]. The high lymphocyte percentages are coincident to that registered in current study, but remaining values are not very comparable because the investigators considered six leukocyte types.

Table 4 detail the physiological variations related to sex and age (liveweight and total lenght) of studied reptiles. Lymphocyte rates from females were significantly higher than those registered in males. Reptile lymphocyte counts are influenced by sex: females of some species have greater lymphocyte number than males [2]. It is affirmed that caiman hematological values register variations attributable to sex [29], although in autochthonous northeastern Argentine specimens other authors did not register significant differences between sexes [27]. White blood cells and heterophil concentrations from adult *Crocodylus palustris* were higher in males than in females [26].

When age progressed from stage 1 (< 3.5 kg liveweight and 100 cm lenght) to stage 3 (> 5 kg and 110 cm), decrease of heterophils and increase of lymphocytes were registered in both grouped species, but differences were not significant perhaps because all animals were sub–adult specimens. In coincidence, heterophils decreased and lymphocytes increased when the age of *C. porosus* advanced (from birth until 2–4 years old), although decreases of total white blood cells concentration were also registered [15].

On the contrary, lymphocyte counts from *C. palustris* were significantly lower in adult than those of both juvenile and sub–adult specimens [26]. In same species, total white blood cells counts were significantly lower in adult than those from both juvenile and sub–adult reptiles, such as eosinophil counts from sub–adult crocodiles were significantly lower than those from both adult and juvenile specimens [26]. It is well–known that mammals physiologically change both total white cells count and leucocytary formula, when age progresses [3]. Variations of leukocytal parameters related to age were not registered by other authors on *C. latirostris* and *C. yacare* [27].

Table 5 expose caiman physiological variations related to year season and handling system. Total white blood cells concentration was significantly lower in cold seasons than in warm ones. This change was caused by the significant decrease of lymphocytes, in spite of the significant increase of monocytes. In agreement, it is affirmed that highest number of crocodile lymphocytes occurs in summer and the lowest in winter, as a result of a decrease in splenic and circulating cells due to hibernation [2]. Descents of *C. porosus* corporal temperature (from 32 until 28ºC) produced corticosterone decrease and significant decreases in total white cell and lymphocyte counts; an increase in these counts was registered when temperature was returned to 32ºC [28]. Because reptiles show a seasonal decline in lymphocyte population during the winter, one would expect the immune responses to be suppressed or inhibited during low temperature or during hibernation [2].

On contrary, reptile monocytes show little seasonal variation [4]. Greater reptile heterophils proportion occurred during summer in other crocodiles [4] was not verified in present study. There appears to be little seasonal variation in the reptile basophil count [23]. The number of eosinophils from other crocodiles would be influenced by seasonal factors where the lowest numbers occur in the summer and highest during hibernation [4]. Although hematological values from autochthonous caimans would fluctuate according to environmental temperature changes [29], authors did not register leukocytal differences among year seasons, neither *C. latirostris* nor *C. yacare* [27].

Caiman restraining system, tanks size, animals/m^2, existence of roof, food type and feeding frequency, such as water type, temperature and renovation frequency, were different in the hatchery and the zoo. However, these different handling systems were not reflected in significant differences on caimans leukogram. It is described that hematological values from reptiles vary due to feeding system [29]. Winter depletion of tissular reserves can cause malnutrition [25], and crocodile lymphocytes are influenced by nutritional status: a decrease in lymphocyte counts occurs during malnutrition [2].

The marked heterogeneity among data obtained in early quoted studies, are probably due to the use of different blood extraction techniques and laboratory analysis, such as factors as sex, age, handling and feeding of reptiles. Several investigators obtained blood by cardiac puncture [7, 8, 24]; in certain cases cardiac blood reveals different values to those from peripheral blood [10]. Some researchers used caudal vein puncture [27]. Others used heparin as anticoagulant [5, 29]; heparin creates a blue tinge to blood films and causes clumping of the leukocytes [2]. In certain rehearsals the sample number was very low, standard deviation overcomes to arithmetic mean, postprandial effect and circadian rhythm were not kept in mind, and it is deduced that it lapsed much time between extraction and blood analysis. Moreover, in some studies the used laboratory methods are not mentioned, the animals were not divided according to sex, age, year season neither feeding system, and the numeric values were published without their corresponding units.

On the other hand, it should be kept in mind that inappropriate restraining maneuvers cause stress in crocodiles [15], and stress is able to markedly alter the leukogram [3]. Few hours after capture, corticosterone of *A. mississippiensis* reveals an increase due to stress [12]; in mammals this hormone causes increase of blood leukocytes and neutrophils, as well as decrease of lymphocytes and eosinophils [3]. Also, reptilian leukocytes suffer a degranulation due to the stress (especially in eosinophils and basophils); this accident can cause leukocitary formula interpretative errors [23].

Hematological data are useful to evaluate physiological state and to detect early the appearance of reptile illnesses [29]. Leukogram can be used to detect and assess such conditions as infection, inflammation, parasitemia, hematopoietic neoplasia, and other blood and organic crocodile disorders [2]. Lymphocytosis can indicate the resolution or passage to chronicity of the inflammatory process [18]. Hematologic data provides clues to the existence of conditions that affect the cellular components of reptilian peripheral blood [2], but they should be obtained with appropriate techniques and they should be interpreted by comparison to those selected from an appropriate reference interval [3].

Nowadays, it is demonstrated that crocodile white cells provide a more effective defense that human leukocytes; it explains the quick cure of their wounds in a highly polluting habitat. Some investigators foresee that the knowledge of such defensive mechanisms could be applicable in the fight against the virus of human acquired immune deficiency syndrome (AIDS) [1].

In conclusion, leukocytal reference values from captive *C. latirostris* and *C. yacare* sub–adults healthy specimens, such as significant physiological variations related to species, sex, age (liveweight, dimensions), environmental temperature (year season), and handling system (housing, feeding), are established. Obtained data can be used to improve the caimans rearing in Argentine northeastern.

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