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Cytomorphology and Seasonal Hematological Parameters in Tegu Lizards (*Salvator merianae*) Raised in Captivity

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Authors' contributions

This work was carried out in collaboration between both authors. Author SNC designed the study, wrote the protocol, did the experimental work and the data collection and wrote the draft of the manuscript. Author OEAA performed the statistical analysis, managed the analyses of the study and collaborated in the writing of the final version of the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: In this study, we describe cytomorphology and haematological values in *Salvator merianae* lizards bred in captivity, in order to establish reference values for health monitoring by veterinarians in animal production.
Study Design: Blood samples obtained from the tail vein of adult specimens at different times of the seasonal cycle were analysed. Cell morphology was characterised by light and electron microscopy and the corresponding blood counts were performed. Seasonal changes and annual averages of hematocrit, haemoglobin, erythrocytes, leukocytes, platelets and percentage leukocyte formula were determined. In addition the blood was analysed by flow cytometry.
Place and Duration of Study: The study was conducted in El Manantial, Tucumán, Argentina with *Salvator merianae* lizards, raised at the experimental farm of the Facultad de Agronomía y Zootecnia of the Universidad Nacional de Tucumán between 2014 and 2017.

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Methodology: Routine methods of haematology adapted to nucleated erythrocytes were used for this study. Five males and five females individually identified were sampled throughout the annual cycle.
Results: Light microscopy showed 7 cell types: erythrocytes, neutrophils, azurophils, basophils, monocytes, lymphocytes and thrombocytes. Flow cytometry was used because it allows an accurate count of the number of white blood cells; an average of 7.2 $x10^3$/µl was obtained. Changes related to seasonality were observed in the hematocrit and haemoglobin. Significant differences in the annual average hematocrit and haemoglobin between the sexes were found with 42.5 ± 0.61% for males and 40.1 ± 0.60% for females. No seasonal changes in the values of leukocytes or the leukocyte differential count were found.
Conclusion: This study provides haematological reference values necessary for an adequate sanitary evaluation of farm-raised *S. merianae.*

Keywords: Hematic cytomorphology; hematic biometry; seasonal changes; reptile.

1. INTRODUCTION

The genus *Salvator* (ex *Tupinambis*, Squamata: Teiidae) whose taxonomic situation was recently revised [1], comprises a group of large South American lizards [2] that exhibit a widespread distribution, covering tropical to temperate climates, where *Salvator merianae* (Tegu lizard) and *Salvator rufescens* are the southernmost representatives [3]. These species of large lizards, up to 1.5 m in length with a body weight of 7 kg, were traditionally used by indigenous communities as a source of meat, fat, and leather [4,5]. Later in the 90's, extensive poaching of these lizards for their leather promoted captive breeding to achieve sustainable use and protection of natural populations [6,7]. In subtropical and temperate environments, *Salvator*, like other ectothermic reptiles and amphibians exhibit a characteristic pattern of alternating periods of activity and hibernation. Hibernation extends about six months (autumn-winter), and during this period the animal remains inactive and does not feed, while, in contrast, during the active stage (spring-summer), it concentrates the functions of development, reproduction and accumulation of reserves with consistent hematologic, metabolic and endocrine changes [4,8,9]. Further studies in this species show seasonal morpho-functional adjustments in the heart of juvenile tegu lizards [10] and changes in the small intestine according to energy demands in response to seasonal variation [11]. Recently, some researchers have described that *S. merianae* has a certain endothermic potential, with a rising in their nocturnal body temperature of 10°C above the ambient temperature during the breeding season [12].

Captive breeding depends on a thorough knowledge of the biology of these lizards to optimise their development and improve their sanitary control. Blood is the most studied fluid in the body of vertebrates because the information provided is important and diverse and it is relatively easy to collect samples. Changes in cytomorphology and haematological values have important implications for animal health. Numerous hematological studies on reptiles exist in the literature [13,14,15,16,17,18,19]. There are also studies in *Tupinambis* [20,21,22,23,24,25]. However, these studies show large discrepancies between the evaluated parameters and the cytomorphological characterisations are not yet sufficiently defined, in addition they were carried out mainly in wild animals, without individual identification and without systematic data throughout the annual cycle.

In this study, we describe cytomorphology and haematological values throughout the annual cycle in *S. merianae* lizards bred in captivity, in order to establish reference values for veterinarian monitoring health in animal production.

2. MATERIALS AND METHODS

2.1 Animals and Hatchery Management

The study was conducted in El Manantial (26°51'S and 65°17'W), province of Tucumán, in Northern Argentina from 2014 to 2017. Adult specimens of *S. merianae*, raised in the experimental farm of Facultad de Agronomía y Zootecnia of the Universidad Nacional de Tucumán were used.

This region is characterised by a temperate/subtropical climate with a dry season during the winter (June, July and August) period. The animals are housed in outdoor enclosures surrounded by masonry walls (1.2 m tall) and equipped with shelters and shade. They are provided with water and food *ad libitum* with a previously developed special farm diet, which consisted of about 85% ground chicken heads and feet (1:2), 15% soybean meal, 0.25% vitamin-mineral supplement for broilers (Micromix^{TM}, Biofarma, Córdoba, Argentina), 0.25% sodium chloride, and 0.1% butyl hydroxy toluene [26]. Based on our previous experiences, these conditions are suitable for animal welfare.

2.2 Experimental Design and Sampling

Adult specimens of both sexes, clinically healthy, between 3 and 8 years of age, weighing 2.5 to 6.2 kg, and measuring 29 to 52.5 cm in snout-vent length were sampled between 2014 and 2017. For hematic cytomorphology studies, 150 smears of 45 animals were examined throughout that period. Seasonal changes in blood parameters were studied in a group of 10 animals, 5 males and 5 females, identified by subcutaneous implantation of a microtransponder (ID-100A, Trovan Electronic Identification. Rosenbusch. Buenos Aires). Hematocrit was determined during three annual cycles and the rest of the haematological parameters were evaluated over one annual cycle.

Blood samples (approx. 1.2 - 1.5 ml) were collected from the tail coccygeal vein, without sedation, using heparinised syringes at specific times of the annual cycle of *S. merianae*: hibernation (Hib), hibernation emergence (HE), reproductive stage (R), post-reproductive stage (PR) and prehibernation (PH). The reproductive period extended from the start of sexual interactions until egg hatching. The post-reproductive stage (reproductive quiescence) was characterised by sexual inactivity and continued until prehibernation, when activity and food intake decrease, in fall, prior to hibernation.

The samples for hematic cytomorphology studies were analysed with optical and electronic microscopy and flow cytometry. The haematological parameters determined were: hematocrit (PCV), haemoglobin (Hb), erythrocytes or red blood cells (RBC), leukocytes or white blood cells (WBC), thrombocytes (Thr), percentage count leukocytes and haematimetric indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

2.3 Hematological Parameters

The PCV was determined by the microhematocrit technique. Cell counts were performed manually with a Neubauer hemocytometer using the Natt and Herrick solution [27]: NaCl 3.88 g; Na_2SO_4 2.50 g; Na_2HPO_4 1.74 g; KH_2HPO_4 0.25 g; Formalin (37%) 7.5 ml; Methyl violet 0.10 g per liter. Haemoglobin values were measured with the cyanmethaemoglobin method, after centrifugation to remove cell debris prior to spectrophotometric reading. The haematimetric indices: MCV, MCH and MCHC were calculated according to the original Wintrobe formulae [28].

2.4 Microscopic Studies

Smears for light microscopy were made immediately with the blood extracted. They were air dried, stained with May-Grünwald-Giemsa [29] and analysed with oil objective on an Olympus BH2 microscope equipped with an Olympus CD35 photographic camera. The cell dimensions were measured using an ocular micrometer. Supravital staining (in fresh erythrocytes) was performed with Cresyl Brilliant Blue solution (Brilliant Cresyl Blue solution - Merck 101384).

For electronic microscopy, the blood was centrifuged, separated buffy coated and fixed in half-concentration Karnovsky's solution [30] (2.5% glutaraldehyde, 2% formaldehyde, in sodium cacodylate buffer at pH 7.4; for 48 hours. The studies of electron microscopy were carried out in the LAMENOA (Laboratorio de Microscopía Electrónica del Noroeste Argentino). Samples for transmission electron microscopy (TEM) were post-fixed in 1% osmium tetroxide, dehydrated in ethanol/acetone and embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate [31] and examined with a Zeiss EM 109 electron microscope. Samples fixed for scanning electron microscopy (SEM) were dehydrated in ethanol/acetone and critical point dried. Samples were sputter-coated with gold and observed with a JEOL CF 35 scanning electron microscope.

2.5 Flow Cytometry

Flow cytometry is a technology that is used to analyse the physical and chemical characteristics of cells in a fluid as it passes through the laser.

The acquisition and analysis of the results were performed with a flow cytometer PARTEC PAS II using software Flomax (multiparameter) from Unidad de Citometría de Flujo at the Universidad Nacional de Tucuman. For the study, 1 ml of heparinised blood from each animal was taken during the reproductive stage of the latest annual cycle. The samples were analysed according to forward scatter (FSC) cell size and side scatter (SSC) intracellular complexity.

The blood samples were examined in normal conditions (unlysed) since lysis performed by FACS (BD FACS™ Lysing Solution 10X Becton, Dickinson and Company) polluted the sample with the released nuclei of erythrocytes.

2.6 Statistical Analysis

We used a mixed models approach to analyse the data [32]. We considered sex, stage and the stage x sex interaction as fixed effects. The random component of the model was selected by means of likelihood ratio tests. The decision about the significance of the different effects was based on the corresponding F tests. Hematocrit means of sex within stages were compared with orthogonal contrasts (1 degree of freedom F tests). We ran these models using the R software and the graphical interface to R included in Infostat [33].

3. RESULTS

3.1 Hematic Cytomorphology

Morphological examination by light microscopy of *S. merianae* blood elements revealed the presence of 7 cell types: erythrocytes, heterophils, basophils, azurophils, lymphocytes, monocytes and thrombocytes. In addition, electron microscopy studies provided ultrastructural characteristics of these cells.

3.1.1 Erythrocytes

They are the most numerous cell type. They are oval or elliptical cells with an average size of 16.1 ± 1.3 µm major diameter and 8.2 ± 0.8 µm minor diameter and 2.1 ± 0.2 µm thick. They have an elongated or oval central nucleus with clumped condensed chromatin and homogeneous cytoplasm (without granulation). When stained with May Grunwald-Giemsa, the core is dark violet and the cytoplasm is gray (Fig. 1A-E). Erythrocytes show a slight anisocytosis, accentuated at the exit of hibernation. In this period, with the supravital dyes, erythrocytes are observed in different stages of maturation with a greater presence of immature cells. The latter are smaller than the mature cells, and exhibit a high nuclear-cytoplasmic ratio, less condensed nuclear chromatin and increased cytoplasmic basophilia. Cresyl Brilliant Blue staining shows greater crosslink density (RNA and ribosomes) in younger cells (Fig. 1F). A few mitotic images were also found in peripheral blood.

Erythrocytes observed with the scanning electron microscope (SEM) are flattened oval discs that allow observation of the slight protrusion of the nucleus in the central region of the cell (Fig. 2A).

Examination by transmission electron microscopy (TEM) shows a nucleus with scant euchromatin and marginal distribution of heterochromatin against the nuclear envelope. The cytoplasm is electron dense and very homogeneous (Fig. 2B).

3.1.2 Heterophils

They are the most numerous leukocytes in the blood of *Salvator* and are homologous to neutrophils in the mammalian blood cells. They have an average size of 16.5 ± 1 µm. They have a lobed nucleus, often with 2-3 lobes of chromatin condensed into lumps and wide cytoplasm covered by large and multiform granules. The granules are elliptical, oval, rounded or rod-shaped, orange-brown and variable in size (Figs. 1A-B, D). When examined with TEM, they have a nucleus with central euchromatin and peripheral heterochromatin. In the cytoplasm, many specific granules of different size, shape and electron density, and nonspecific granules of smaller size and lower electron density are observed. They also have an extensive rough endoplasmic reticulum (RER), Golgi and few mitochondria. On the cell surface some filiform extensions are observed (Fig. 2C).

3.1.2.1 Eosinophils

In blood smears of lizards *Salvator* the eosinophils are indistinguishable from heterophils by conventional staining. Only one fragment of cytoplasm containing granules characterised by a central crystalloid was found in the slices of electron microscopy (Fig. 2D). In relation to the other cell types, they were found in a very low proportion.

3.1.3 Basophils

These are cells of variable size (9.6 ± 1.1 µm) with a round, bi or trilobed nucleus. The weakly basophilic cytoplasm and nucleus are covered by a dense, heterogeneous, dark and metachromatic granulation. The typical granulation produces a scalloped or irregularly edged cell with an appearance similar to the mammalian basophil (Fig. 1A). With TEM, cytoplasmic granules of different sizes and electrodensities are observed, predominantly of electron-dense granules. Some organelles, such as Golgi apparatus, mitochondria and cisterns of the rough endoplasmic reticulum are seen.

3.1.4 Azurophils

Azurophils, with an average diameter of 15.2 ± 2.9 µm, have a large nucleus, round or oval of loose chromatin, located eccentrically. The extensive cytoplasm (neutral or weakly basophilic), is covered with an azurophilic granulation, densely clustered on the periphery of the cell and less concentrated in the perinuclear region (Fig. 1B). The cytoplasm is occasionally vacuolated. Ultrastructurally they show a euchromatic nucleus with heterochromatin distributed in a few lumps. The cytoplasm contains abundant small granules of varying density. Dispersed glycogen granules and a moderate number of organelles such as Golgi, mitochondria and endoplasmic reticulum are seen.

3.1.5 Lymphocytes

Lymphocytes are generally the smallest leukocyte cells, similar to the thrombocytes, and show variations in diameter with an average of 6.8 ± 1.1 µm. They have a round central nucleus of condensed chromatin and scant basophilic cytoplasm (Fig. 1C). Some of the large lymphocytes occasionally exhibit thick cytoplasmic azurophilic granules. With MET we observed a round central nucleus with predominant euchromatin, peripheral heterochromatin, rough endoplasmic reticulum, mitochondria and few cytoplasmic granules.

3.1.6 Monocytes

These cells are between 12 and 18 µm (15 ± 2.4 µm average) and have an unusual appearance with an eccentric, bilobed, round or reniform nucleus and generally clumped chromatin. They exhibit a cytoplasm, weakly basophilic, with fine azurophilic granules and occasionally toxic granulation and vacuolation (Fig. 1D). In some animals, these cells showed a cytoplasmic hyaline inclusion with a ring or crescent-shaped appearance (Fig. 1E) that could not be identified. Ultrastructurally, this inclusion was electron-lucida and the cells showed fine cytoplasmic granulation and a nucleus with heterochromatin located peripherally.

3.1.7 Thrombocytes

They are small oval cells with an approximate major diameter between 6 and 8 µm, (7.2 µm an average) with a round to oval nucleus of condensed chromatin. They have a high nucleus-cytoplasm ratio and scant basophilic cytoplasm with prominent vacuoles in one or both cell poles (Figs. 1B-C). The TEM examination showed a complex nucleus with abundant indentations. The cytoplasm exhibits an important canalicular system, microtubules, large vacuoles, mitochondria and cytoplasmic threadlike prolongations (Fig. 2F).

3.2 Hematic Biometry

Captive Tegus showed variations in some haematological parameters associated with stages of the seasonal cycle and diferences linked to sex. In fact, the levels of the hematocrit in *S. merianae* throughout the annual cycle exhibit changes associated with seasonality. The hematocrit showed high values during hibernation that decreased significantly at the exit of hibernation. At this point the active stage of the animals begins and an increase in the hematocrit is observed during the reproductive period while, in the reproductive quiescence stage the hematocrit reaches the minimun absolute values to rise up again in the pre-hibernation (Fig. 3).

We observed significant differences between males and females in the annual average hematocrit and in specific points of the cycle (Fig. 4). Although both sexes presented the same profile throughout the annual cycle, the males, in all the stages, exhibited higher values than the females (Fig. 4) reaching significant differences only in the stages of hibernation, post-productive period and pre-hibernation.

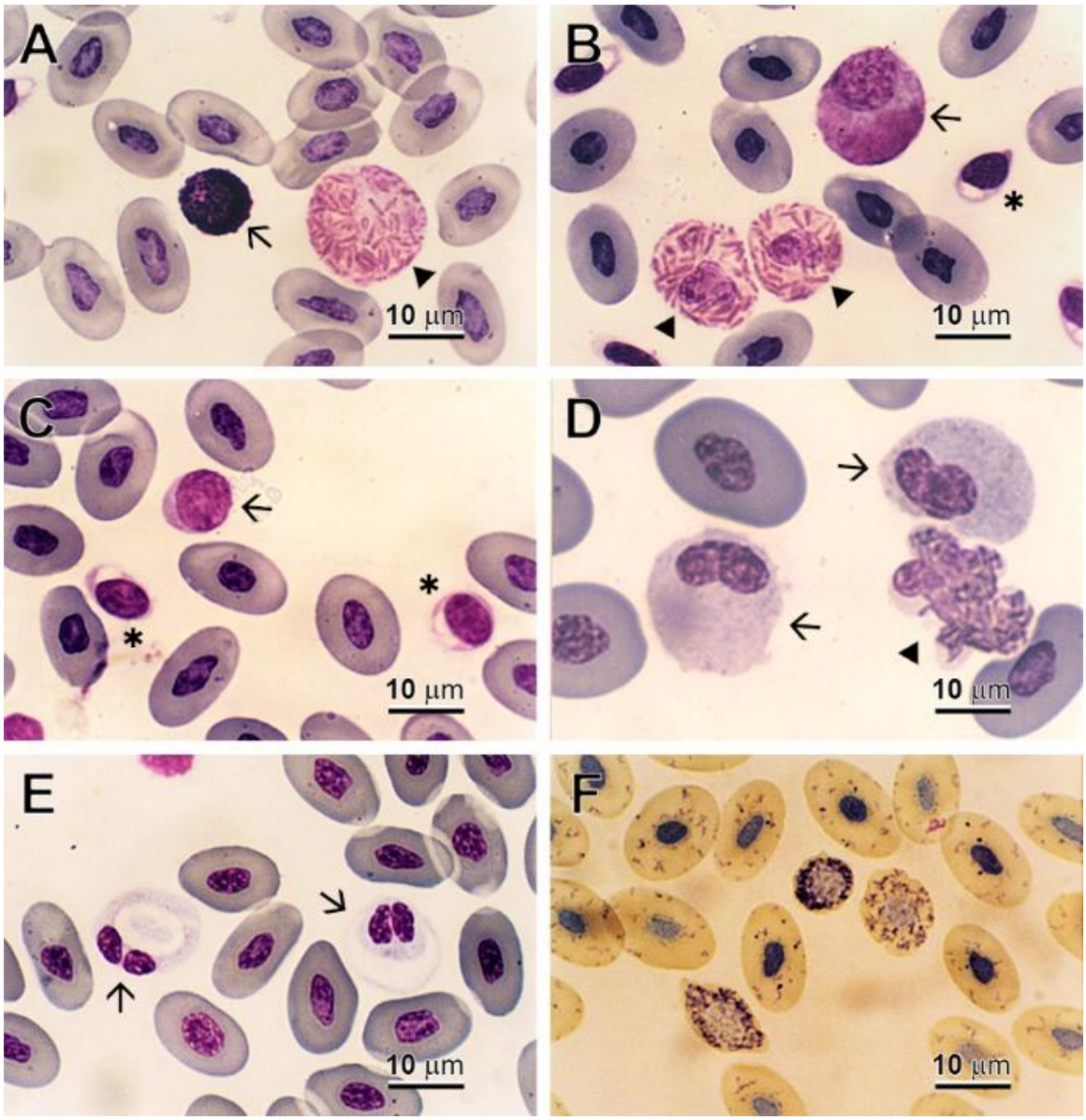

Fig. 1. Blood cells in *Salvator merianae.* Light microscopy.
A: Erythrocytes; basophil (arrow) and heterophil (arrowhead) B: Erythrocytes, azurophil (arrow), two heterophils (arrowhead) and two thrombocytes (asterisk); C: Erythrocytes, lymphocyte (arrow) and two thrombocytes (asterisk); D: Erythrocytes, two monocytes (arrow) and a deformed heterophil (arrowhead); E: Monocytes with cytoplasmic inclusion (arrow), F: Reticulocytes, different degrees. Figs. 1A-E: stain May Grunwald Giemsa. Fig. 1F: Briliant Cresyl Blue.

The number of erythrocytes and the hematimetric indices in both sexes showed slight changes throughout the seasonal cycle, without reaching significant differences.

Similarly, the leukocyte and thrombocyte values and the leukogram did not show significant seasonal or intersex differences. The leukocyte count, minimum in the period of hibernation, increased slightly during the active stage of the animal without showing significant differences between the stages of the annual cycle. Since values in all animals were similar and the changes throughout the annual cycle were not significant, we grouped the individual results as male and female annual means with lower and upper confidence limits (Table 1).

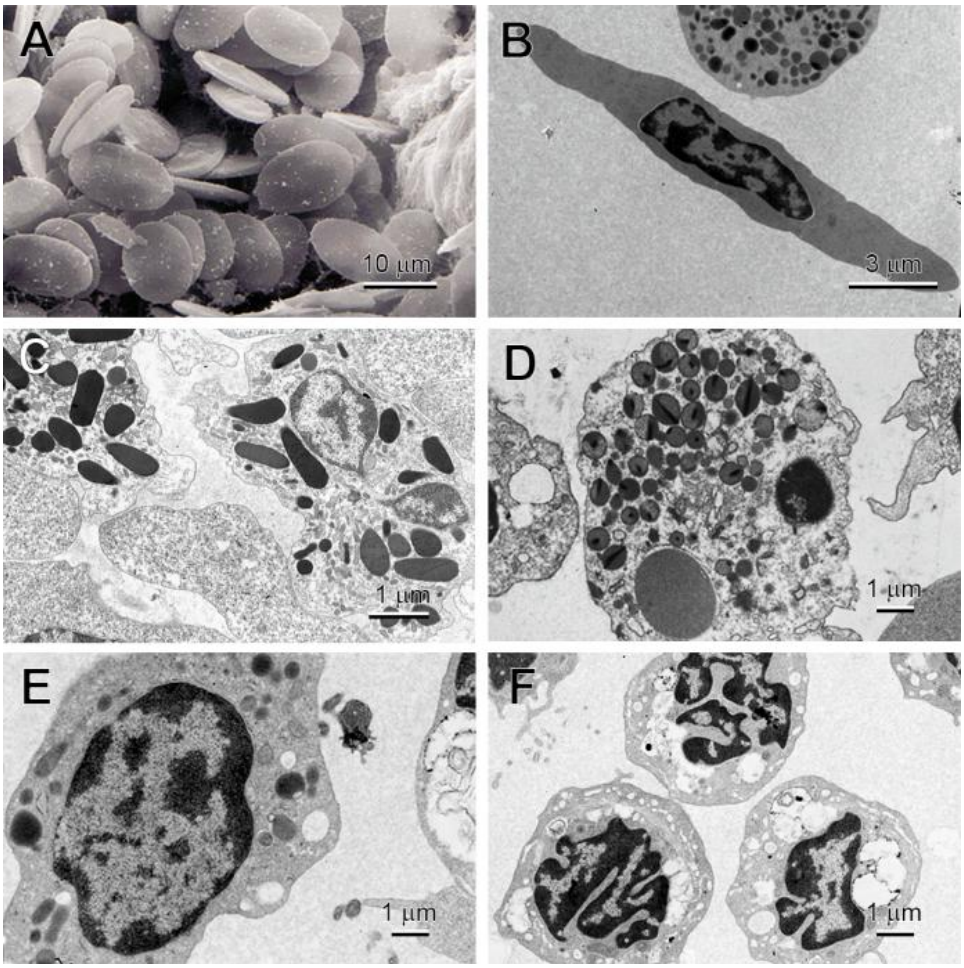

Fig. 2. Blood cells in *Salvator merianae.* Electron microscopy.
***A**: Erythrocytes (SEM); B: Erythrocytes (TEM); C: Heterophils (TEM);*
D: Cellular fragment with eosinophilic granulation; (TEM); E: Lymphocyte (TEM); F: Thrombocytes (TEM).

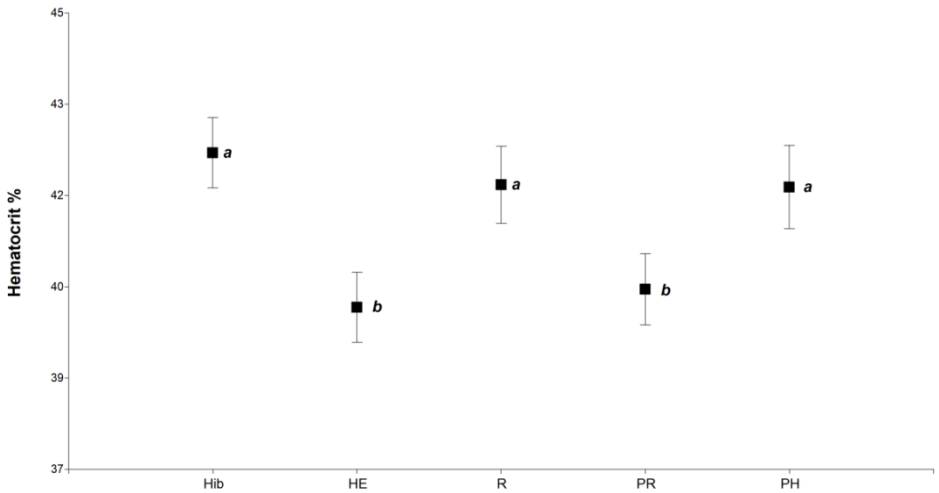

Fig. 3. Mean hematocrit values of captive *Salvator merianae* throughout the annual cycle
Hib: hibernation; HE: hibernation emergence; R: reproductive stage; PR: post-reproductive stage; PH: Pre-hibernation.
Different letters indicate significant differences according to Fisher´s test (P <.001).
Mean values ± Standard error of means. (5 males and 5 females measured during three annual cycles)

3.3 Flow Cytometry

Flow cytometry examination of blood samples allowed us to differentiate three populations: R1- erythrocytes, R2- lymphocytes plus thrombocytes, and R3- the remaining leukocytes (Fig. 5), according to FSC dispersion parameters (cell size) and SSC (intracellular complexity).

In addition, this technique allowed us to obtain the leukocyte number in relation to the red blood cell number. The leukocyte population, in the average sample, corresponded to 0.73% of acquisitions (2724 events / 372166 events), what in an average sample of 980000 erythrocytes/µl corresponds to 7154 leukocytes/µl.

This technique did not allow us to discriminate the leukocyte types. Moreover, we tried to characterise the leukocytes using human antibodies with unsuccessful results.

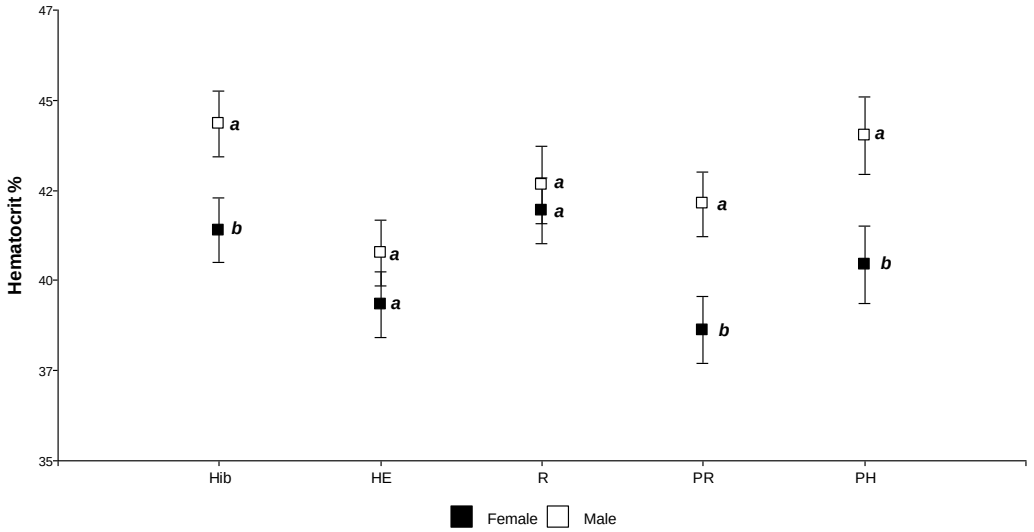

Fig. 4. Mean hematocrit values of *Salvator merianae* lizards throughout the annual cycle discriminated by sex.

Hib: hibernation; HE: hibernation emergence; R: reproductive stage; PR: post-reproductive stage; PH: Pre-hibernation.

Different letters indicate significant differences between sexes within each stage using orthogonal contrasts (P values = .02, .27, .62, .001, .02).

Mean values ± Standard error of means. (*males and 5 females measured during three cycles)*

Table 1. *Salvator merianae* annual averages of blood parameters in males and females

	Males (n = 25)	**Females (n = 25)**	***P***
Erythrocytes 10^6/µl	1.06 (0.96, 1.16) [a]	1.00 (0.92, 1.08) [a]	.62
Hematocrit %	42.5 (41.4, 43.5) [a]	40.1 (38.9, 41.3) [b]	.01
Hemoglobin g/dl	13.1 (12.0, 14.1) [a]	12.1 (11.31 12.9) [b]	.01
MCV fl	398.1 (366.4, 429.8) [a]	399.9 (372.1, 427.8) [a]	.37
MCH pg	124.7 (115.3, 134.1) [a]	119.8 (111.8, 127.9) [a]	.62
MCHC g/dl	30.9 (29.2, 32.6) [a]	29.0 (27.6, 30.5) [a]	.62
Leukocytes 10^3/µl	7.0 (6.0, 8.1) [a]	7.3 (5.8, 8.7) [a]	.37
Heterophils %	53.5 (48.2, 58.0) [a]	50.4 (43.9, 56.9) [a]	.47
Lymphocytes %	21.4 (16.7, 26.1) [a]	22.5 (16.0, 29.0) [a]	.75
Monocytes %	7.5 (5.2, 9.9) [a]	8.1 (4.8, 11.4) [a]	.22
Basophils%	6.8 (3.5, 10.1) [a]	8.7 (6.0, 11.4) [a]	.56
Azurophils%	10.9 (8.7, 13.1) [a]	10.5 (8.3, 12.7) [a]	.14
Thrombocytes10^3/µl	28.7 (25.4, 32.0) [a]	29.3 (22.8, 35.8) [a]	.67

Mean (95% lower and upper confidence limits). Different letters indicate significant differences

MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

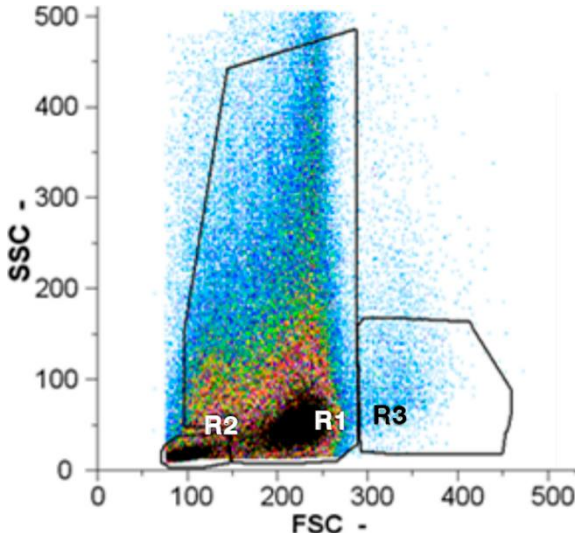

Fig. 5. Flow cytometry of whole blood of *Salvator merianae*. Cell populations
Note the differentiation of three populations: R1- erythrocytes, R2- lymphocytes + thrombocytes and R3- remaining leukocytes.

4. DISCUSSION AND CONCLUSIONS

The development of productive hatcheries of *Salvator* lizards and the rise of mascotism of these reptiles in the world requires precise hematological parameters for an adequate veterinary management.

There is general agreement about erythrocyte morphology in reptiles, with certain differences in the size and number of cells in different species. In *Salvator* erythrocytes, there were practically no differences in morphology, size and number of red blood cells compared to previously reported values [20,21,24,25]. However, throughout the annual cycle we found slight morphological variations: in particular, a degree of anisocytosis at the exit of hibernation. In this same period, in coincidence with the description by Carvalho et al. [23], we found increased numbers of reticulocytes associated with erythrocyte regeneration, although few mitotic figures in peripheral blood were seen.

These studies in captive Tegu lizards showed changes in hematocrit during the annual/seasonal cycle and also differences between the sexes. The highest values were found during hibernation, in agreement with Pena et al. [21] and Troiano et al. [24,25] who explain the findings as a result of dehydration with hemoconcentration and increased levels of erythropoietin in response to hypoxia. They did not find changes linked to sex. In *Salvator* our studies revealed significant differences in the hematocrit annual average, with values higher in males than in females (similar to that found by Arguedas et al in the lizard *Microlophus bivittatus* [34]). Values analysed throughout the annual cycle for males and females separately show the same pattern of variation, although females generally exhibit lower hematocrit values. The minimum absolute values are in the post reproductive stage. This finding is probably related to activities carried out by females that oviposit and remain for 70-80 days in the nest incubating eggs with characteristic maternal behaviors [6,35,36,7]. In contrast, Harr et al. [37] reported significantly higher hemoglobin concentrations, PCV and MCHC in adult female green iguanas compared with their male counterparts. Pejrilová et al. [38], in the same species, did not find significant seasonal or sexual differences in hematological parameters, although he found differences associated with age.

In reptiles, some leukocytes differ morphologically from those described in mammals and different nomenclature is used to classify them. There are also discrepancies in their characterisation [14,17]. Flow cytometry failed to characterise the different types of leukocytes in *Salvator* but allowed us to establish populations of erythrocytes, leukocytes and platelets, matching those reported by Inoue et al. [39] in green iguana (*Iguana iguana*).

In general, the different types of leukocytes (heterophils, azurophils, lymphocytes, basophils) and thrombocytes in *S. merianae* have morphological features which allow us to identify them unequivocally.

However, identification of eosinophils using routine colorations was inconclusive. This was only possible with electron microscopy which allowed us to observe (in a section) a portion of cytoplasm containing granules with a central crystalloid. This image is similar to that found by Carvalho et al. [23] in *Tupinambis'* eosinophils. The low incidence of eosinophils in electron microscopy cuts analysed coincides with that reported by Harr et al. [37] who found 0.7% eosinophils in green iguana. Eliman [40] does not mention these cells in the blood of the lizard *Pogona vitticeps*.

The most striking finding in *Salvator* blood was associated with monocytes. These cells have

been described in the literature, but with a different morphology from what we found. The monocytes showed a segmented (bilobed) eccentric nucleus and basophilic cytoplasm (frosted appearance) with fine submicroscopic granulation (Fig. 1D). Monocytes contained, in several specimens, an intracytoplasmic hyaline inclusion, which was ring or crescent shaped and whose nature could not be established (Fig. 1E). Similar findings reported in the literature in the cytoplasm of lizard and snake erythrocytes were identified as haemoparasites (Piroplasmids and haemogregarines) [14,41].

In many haematological studies of reptiles the main difficulty lies in determining the number of leukocytes in the presence of nucleated red blood cells [14]. This explains the leukocytosis reported in some cases.

Studies using flow cytometry allowed us to determine accurately the number of white blood cells, which in our case coincided with the manual count (using Natt and Herrick reagent). The average value obtained for *S. merianae* (7200 cells/µl) is similar to that reported by Pena et al. [21] and differs markedly with the 16700 found by Troiano et al. [24,25] for the same species.

We found significant differences in the differential leukocyte count with respect to that reported in the same species by Pena et al. and Troiano et al. (*op cit*.). They found a large number of eosinophils (42% and 24%) with few heterophils (they probably confused heterophils with eosinophils, given their similar morphology). In their paper, Troiano et al. [24,25] reported 45% lymphocytes, which could be due to the involuntary count of thrombocytes and nuclei-free erythrocytes. In general, all the bibliography agrees that the round thrombocytes may be confused with small lymphocytes [19], which possibly explains the great lymphocytosis observed in some cases in reptiles.

The aforementioned difficulties in the leukocyte count, the particular morphology of the heterophils and the confusion of platelets with lymphocytes are ambiguities that can make the evaluation of the reptile hemogram difficult [17].

It should be emphasised that an adequate knowledge of haematological parameters permits the detection not only of changes in the health status of the farm population, but is also widely used, in vertebrates, as a stress sensor (number and profile of leukocytes) [42] or of environmental changes or anthropogenic threats in natural populations [34,43].

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws. All experiments have been examined and approved by the ethics committee of Consejo de Investigaciones of Universidad Nacional de Tucumán (CIUNT).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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