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Baseline values of immunologic parameters in the lizard *Salvator merianae* (*Teiidae*, Squamata)

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Abstract
The genus *Salvator* is widely distributed throughout South America. In Argentina, the species most abundant widely distributed is *Salvator merianae.* Particularly in Santa Fe province, the area occupied by populations of these lizards overlaps with areas where agriculture was extended. With the aim of established baseline values for four immunologic biomarkers widely used, 36 tegu lizards were evaluated tacking into account different age classes and both sexes. Total leukocyte counts were not different between age classes. Of the leucocytes count, eosinophils levels were higher in neonates compared with juvenile and adults; nevertheless, the heterophils group was the most prevalent leukocyte in the peripheral blood in all age classes. Lymphocytes, monocytes, heterophils, azurophils and basophils levels did not differ with age. Natural antibodies titres were higher in the adults compared with neonates and juveniles lizards. Lastly, complement system activity was low in neonates compared with juveniles and adults. Statistical analysis within each age group showed that gender was not a factor in the outcomes. Based on the results, we concluded that *S. merianae* demonstrated age (but not gender) related differences in the immune parameters analyzed. Having established baseline values for these four widely-used immunologic biomarkers, ongoing studies will seek to optimize the use of the *S. merianae* model in future research.
Keywords: Biomarkers, Immune system, Reptilian, *Salvator merianae*, Sentinel model.

Introduction

The genus *Salvator* (previously *Tupinambis*; Harvey *et al*., 2012) belongs to the order Squamata, and is widely distributed throughout South America. In Argentina, the most abundant and widely distributed species is the "iguana overa (Spanish) or tegu lizard (English)" (*Salvator merianae*) (Ávila-Pires, 1995; Harvey *et al*., 2012). This lizard is a diet generalist that feeds on a wide range of animals and fruits (de Castro and Galetti, 2004) and whose daily and seasonal activity cycles are strongly related to temperature (Winck *et al*., 2011). From April-June (colder months), their metabolism decreases until environmental temperature increases. From October-December (warmer months), the annual reproductive cycles takes place. In those times, nests are built in caves in the ground or tree roots that are isolated from the climate changes to ensure a proper incubation temperature and humidity for the development of embryos (Yanosky and Mercolli, 1992). Since 1982 *S. merianae* has been included in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and it is considered of "Least Concern" for conservation status by the IUCN (International Union for Conservation of Nature) (Embert *et al*., 2009). In addition, this species has been under management on a sustainable use program in Santa Fe province (Argentina), known as Iguana Project (PI - Secretary of State for Environment and Sustainable Development of the Province of Santa Fe. Resolution Number 0031/07). This program is based on the ranching technique, which implies the collection of eggs from natural environment, subsequent artificial incubation, birth and breeding of the animals under controlled conditions until they reach an appropriate size to be released into the wild and to avoid predation or influence of low temperatures (Schaumburg *et al*., 2012).

In recent years, the habitats of this species have been negatively impacted by expansion of agriculture, especially soybean monocultures. The implementation of a system that combines the use of new technologies includes use/application of chemicals in bulk. The effects of these agents, combined with environmental factors (i.e., habitat fragmentation/degradation, draining of wetlands) have produced a decline in the populations of various wild species, among them

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several types of reptiles (Gibbons *et al*., 2000; Santos and Llorente, 2009; Weir *et al*., 2015). In particular, tegu lizard populations have plummeted in areas where agricultural activity has advanced.

As with any endangered host species, it is essential to monitor their health status and ability to defend from infections, as a result of changes in their external environments. Immunity to infection is mediated by two general systems, innate (or natural) and acquired (or adaptive). Components of the natural immune system are markedly conserved between insects and mammals, indicating a common ancestral origin for this branch of immunity (Hoffmann *et al*., 1999). Only vertebrates have the adaptive immune system, which play an important role when innate immunity is not precise enough to a particular challenge or in response to recurrence of a given challenge (Collado *et al*., 2008). Exposure of native animals to certain pesticides, whether acute or chronic, could affect both their innate and acquired immune responses (Burns *et al*., 1996). Previous studies have shown some pesticides have effects on immunity; they can alter structures of some components of the immune system (IS), and in some cases also reduce host resistance to antigens and infectious agents (Hernández Coronado, 2007; Modesto and Martinez, 2010; Ray *et al*., 2015). Studies in broad-snouted caiman (*Caiman latirostris*), another native reptile, demonstrated alterations in the IS of hatchlings and neonates that had been exposed to glyphosate, endosulfan, or cypermethrin formulations (Siroski, 2011; Harvey *et al*., 2012; Latorre *et al*., 2013; Siroski *et al*., 2016).

White blood cells (WBC) are involved in a significant amount of processes in the IS. In certain situations, increases or decreases in values of select blood components can be used as markers to diagnose disease or changes in host nutritional status (González Fernández, 2003). The innate immune system can be monitored through assessments of two humoral components: levels of natural antibodies (NAb) and the functionality of the complement system (CS). Natural antibodies are encoded directly by the germline genome (Avrameas, 1991) and do not require somatic hyper mutation and recombination during ontogenesis as occurs with the adaptive antibody repertoire. The CS is an important part of the innate immune system and can be sequentially activated in a cascade type reaction by numerous routes (Siroski *et al*., 2016).

The aim of this study was to evaluate four widely-used immune biomarkers, e.g., total and differential leukocyte count, NAb levels, and CS activity, in order to determine baseline values for *S. merianae* of varying ages. This data would, in turn, allow for the potential implementation of this species as a model for use in evaluating exposures of environmentally relevant species to different environmental stressors.

Materials and Methods

Animals

This research was approved by the Ethics Committee and Security (ECAS) of Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral (#258/16, Santa Fe, Argentina). All animals were treated in accord with the Reference Ethical Framework for Biomedical Research: Ethical Principles for Research with Laboratory, Farm, and Wild Animals (NSTRC, 2005).

In this study were used 36 specimens of tegu lizards: 12 neonates (NE; 2-days-old), 12 juveniles (JUV; 6-mo-old) and 12 adults (AD; > 4-yr-old; 6 males, 6 females). The NE and JUV animals came from eggs collected in the Managed Natural Reserve "El Fisco" located in Santa Fe Province, Argentina; corresponding to a Protected Natural Area (Law 12.930, 2008) situated at least 20 km away from possible pesticide application area or other industrial contaminant activity (Siroski *et al*., 2016). As part of the ranching program "PI", the eggs were artificially incubated under controlled conditions of temperature (29-32°C) and relative humidity (< 20%). Adult lizards were taken out from the breeding stock in captivity in the "PI". All lizards were fed *ad libitum* three times a week. The NE and JUV could be sexed when they were below 20 cm in snout-vent length (SVL) (Yanosky and Mercolli, 1992). Therefore, gender determination was performed using the mound scale method as well as through observations of hypertrophy of the jaw muscles in males (Hall, 1978; Yanosky and Mercolli, 1992).

Blood samples

Peripheral blood samples were obtained from the caudal vein (Olson *et al*., 1977) with heparinized syringes (25 G × 5/8″ needles for NE lizards and 1″ needles (21G) for JUV and AD lizards). Aliquots of the collected blood were used for measures of WBC and for differential leukocyte population counts. The remaining sample was centrifuged at 2500 × g for 15 min and stored at -80°C until used for the determination of NAb levels and CS activity (Siroski *et al*., 2016).

Total white blood cells and differential leukocyte population counts

WBC counts were performed using a Neubauer chamber. An aliquot of whole blood was diluted 1:200 with a solution of 0.6% NaCl and then examined under microscope at 400X. All results were expressed as total cells/mm^3 blood (Lewis *et al*., 2008). For the differential counts, two smears were prepared/animal, fixed with ethanol, and then stained with May-Grunwald-Giemsa solution. The preparations were then coded to achieve maximum objectivity during the analysis. Amounts of each leukocyte subtype (e.g., heterophil, basophil, eosinophil, lymphocyte, monocyte, azurophil)/100 WBC analyzed were determined manually using an optical microscope.

Each subtype was expressed in relation to WBC count recorded beforehand. Also, lobularity index was calculated (LI = number of counted lobes / number of heterophils counted) from the classification of heterophilic granulocytes according to Arneth (Charipper, 1928) to evaluate the degree of leukocyte maturity (García *et al*., 1997).

Natural antibodies titres

Determination of agglutinating NAb titres was conducted using a hemagglutination assay described by Matson *et al*. (2005). This assay is based on agglutination between NAb from lizard plasma samples and rabbit red blood cells (RRBC) obtained from a breeding stock maintained at the Universidad Nacional del Litoral. Here, whole rabbit blood was centrifuged at 2500 × g for 15 min to separate the plasma. A buffer solution was then prepared with phosphate-buffered saline (PBS, pH 7.4) containing rabbit plasma (1%) (Sigma, St. Louis, MO). The pelleted RRBC were then washed with PBS several times until the supernatant was clear, and then a 1% RRBC (v/v) solution in PBS was prepared. For the assay itself, to 96-well round (U)-bottom plates (Corning Costar, Corning, NY), 25 µl (PBS with rabbit plasma) was added into wells in Columns 1-12. Thereafter, 25 µl test plasma was added to wells in the first column. Samples were then serially diluted to a final dilution of 1:2048 (Column 11). As a negative control, no lizard plasma was placed into the Column 12 (i.e., well contained only PBS). Finally, 25 µl RRBC solution was added into wells in all columns (1-12). After incubation at 25°C for 1 hr, NAb titres were determined. Titres were assigned as the inverse of the highest dilution yielding a button; in cases where an individual had negative hemagglutination in all wells, a titre of 0 was assigned. From the average of all titres in a given age group, a mean titre for each group was calculated.

Complement System (CS) activity

Lizard CS activity was determined via assessment of sheep red blood cell (SRBC) hemolysis (Siroski *et al*., 2010). The SRBC were collected from Merino sheep (*Ovis aries*) with heparinized syringes. The blood was washed with PBS several times until the supernatant was clear, and then a 2% SRBC (v/v) solution in PBS was prepared. In the assay, lizard plasma was incubated with an equal volume of 2% SRBC for 30 min at 25°C and then centrifuged at 2500 x g for 5 min. Thereafter, 300 µl of the resultant supernatant was transferred to a microplate for measure of optical density [at 540 nm] in a Multiskan RC microplate reader (Multiskan Labsystem, Helsinki, Finland). As a positive control, 2 µl Triton X-100 was added to 1 ml of 2% SRBC and the mixture shaken until complete hemolysis was attained. The level of SRBC hemolysis in each sample was divided by the absorbance of the positive control to obtain the maximum percentage of hemolysis (% MH). All results were expressed as mean % MH [± SE].

Statistical analysis

Data were tested for homogeneity using a Levene test, and for normality using a Kolmogorov-Smirnov test. To determine differences among the groups, data were analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey test. When the data did not meet the assumptions of normality and / or homogeneity of variances, it were analyzed using a non-parametric Kruskal-Wallis test followed by a Mann-Whitney test. Comparisons of variables analyzed as a function of gender were done using a Student *t*-test except for eosinophil data that were analyzed using a non-parametric Mann-Whitney test. Differences were considered significant with $p < 0.05$. All data are reported as means ± SE.

Results

Because the morphological characterization of the leukocytes of this lizard has not yet been reported, we include images of all leucocytes types (Fig. 1).

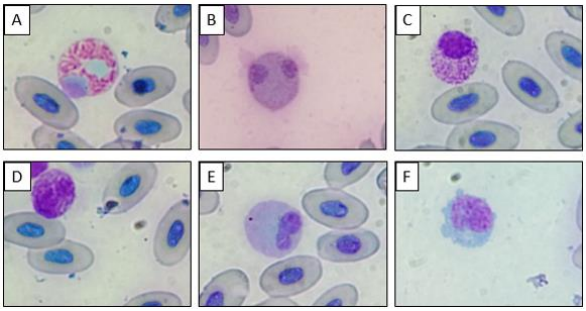

Fig. 1. Leukocytes of *S. merianae*. **(A, B):** Heterophils with cytoplasm full of rod granules **(A)** and of little round granules **(B)**. **(C):** Eosinophil with cytoplasm full of round granules. **(D):** Basophil with big round granules. **(E):** Monocyte and **(F):** Big lymphocyte. In the last two, low nucleus/cytoplasm ratio and low concentration of heterochromatin.

The results for the total WBC counts showed there were no differences among the age classes of *S. merianae.* Nevertheless levels of eosinophils were higher in NE compared with JUV and AD groups (Fig. 2). With respect to the others leucocyte types no differences were observed among age classes. When the mean of all cell types in the three age classes were compared, it was found that the heterophils group was the most prevalent leukocyte in the peripheral blood in all age classes. In this type of leucocyte the nucleus was rounded, with one or two lobes, being infrequent the presence of three or more lobes, determining an IL of 1.76 ± 0.10 for NE, 1.65 ± 0.58 for JUV and 1.61 ± 0.92 for AD. The comparison among age classes was not different. Natural antibody titres differed among the groups. Values associated with AD were higher compared with those for NE and JUV (Fig. 3).

This clearly indicated that the levels of NAb in younger lizards were very low (in many cases, there were titres of 0). With regard to CS activity, the data indicated there were also differences among the age classes. Neonate blood imparted a low value of maximum hemolysis compared with the blood from JUV or AD (Fig. 4). Lastly, comparisons performed for each parameter examined here showed there were no gender-related differences.

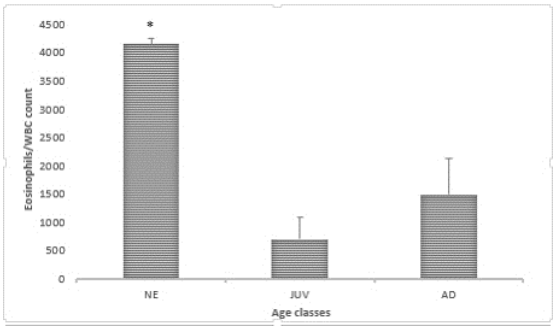

Fig. 2. Eosinophils level in relation with total number of WBC in each age class. NE (neonate), JUV (juvenile), AD (adult). Values shown are means (± SE) from 12 lizards/group. *Value significantly different from other groups ($p < 0.05$).

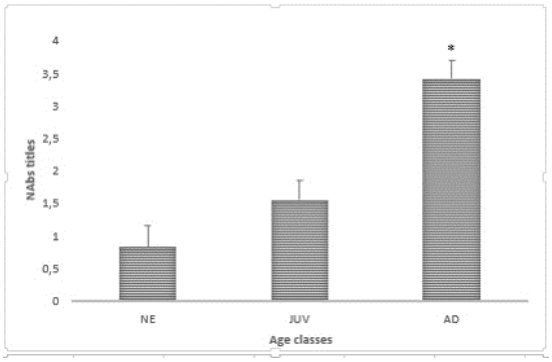

Fig. 3. NAb titres in each age class. NE (neonate), JUV (juvenile), AD (adult). Values shown are mean (±SE) from 12 lizards/group. *Value significantly different from other groups ($p < 0.05$).

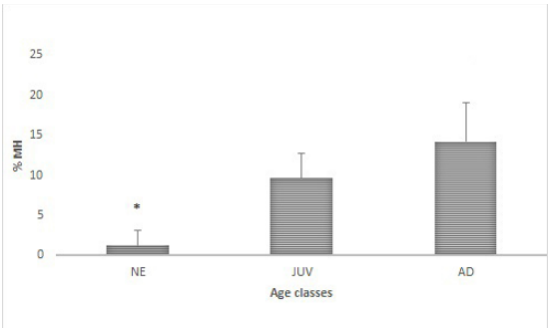

Fig. 4. Maximum percentage hemolysis (%MH) in each age class. NE (neonate), JUV (juvenile), AD (adult). Values shown are means (± SE) from 12 lizards/group. *Value significantly different from other groups ($p < 0.05$).

Discussion

Reptile immunology involves cell-mediated and humoral components; however, knowledge about immune function in reptiles remains limited. Several characteristics of *S. merianae* suggest it could potentially be useful as a sentinel organism for monitoring of environmental agent impact on their habitat (Schaumburg *et al*., 2016).

The present study sought to establish some baseline parameters for such use; in particular, information on the IS of these hosts. Similar results were obtained for other authors who did not find differences in levels of WBC among different age classes in reptiles. Studies on *Chelonoidis chilensis chilensis* (Troiano and Silva, 1998), from captive *Caiman latirostris* and *C. yacare* (Mussart *et al*., 2006) and *Iguana iguana* (Novoa-Fajardo *et al*., 2008) have reported an absence of influence from age on this hematologic parameter. However, Barboza *et al*. (2008) reported values significantly lower in adult than in both juvenile and sub–adult of *C. latirostris*. Further, the present study showed lower WBC counts compared to Novoa-Fajardo *et al*. (2008) results, and higher values in relation to that seen in other studies (Mussart *et al*., 2006; Barboza *et al*., 2008; Silvestre, 2014).

Regarding the endpoint of differential leukocyte counts, here we have considered azurophils as a variation of normal monocytes, as well as some authors (Montali, 1988; Huber and, 1998; Claver and Quaglia, 2009). In contrast, others researches consider them as a distinct cell type (Troiano *et al*., 1996; Kanchanapangka *et al*., 1999; Salakij *et al*., 2002; Maceda-Veiga *et al*., 2015).

Similar to the one reported by Rios *et al*. (2003) for *Lama guanicoe*, in this study a higher level of eosinophils was seen in neonates compared to juveniles and adult lizards. This could be a consequence of some exposures to allergens present in the nest material in contact with neonates. This result contrast with findings by Barboza *et al*. (2008) who observed an age-related increase in eosinophil levels in *C. latirostris.* Overall, the means levels of eosinophils in adult *S. merianae* were generally higher than those reported by Silvestre (2014).

Similar to what was seen by Troiano *et al*. (1996), Troiano and Silva (1998) and and Rios *et al*. (2003), this study did not reveal any differences among age classes in regard to other leukocyte types, while Stacy *et al*. (2011) described age related changes in lymphocyte and heterophils percentages in *Caretta caretta* turtles. The lobularity index here observed was similar to the ones reported by Cabagna Zenklusen *et al*. (2011) in *Rhinella fernandezae* and Salinas *et al*. (2015) in *Rhinella arenarum.* With regard to NAb levels, our analysis of titres showed the highest values in the adults compared with juveniles and neonate

lizards. This increase in NAb with age has been also reported in mammals, birds, and others reptiles (Parmentier *et al*., 2004; Benatuil *et al*., 2008; Sparkman and Palacios, 2009; Ujvari and Madsen, 2011; Zimmerman *et al*., 2013). In reptiles of all ages, the specific antibody response is slower and less robust than in mammalian counterparts (Zimmerman *et al*., 2010). This thus implies that the increase in NAb tires with age might be viewed as a "positive" change in immunity with age in these reptiles (Ujvari and Madsen, 2011). Studies about complement-system activity have been reported with a variety of reptiles. In *Alligator mississippiensis* (Merchant *et al*., 2005), *Crocodylus porosus* and *C. johnstoni* (Merchant and Britton, 2006), and *Caiman latirostris* (Siroski, 2011), potent CS were reported; this suggested a strong physiological importance for this system in terms of protection against various pathogens (Siroski, 2011). In the present study, a low percentage of maximum hemolysis was detected in *S. merianae* compared to the above-mentioned species. This could be a trait of the *S. merianae* itself. On the other hand, differences in CS activity were noted among the *S. merianae* age classes, indicating activity of this system increased with age. Strasser *et al*. (2000) reported similar results in relation to age in mammals. Thus, future studies must consider that results of this technique are likely to be variable with age.According with findings in other studies on sentinel species, the comparisons between sexes revealed no differences in the endpoints assessed here. Khan *et al*. (2016) found no gender-related differences in WBC levels, differential leukocyte counts, or other biochemical/hematological parameters in dogs in Bangladesh. Latorre *et al*. (2015) noted no differences between males and female *Phrynops hilarii* (a side-necked turtle) in any of the variables that were analyzed, including WBC levels and differential leukocyte counts. In contrast, Barboza *et al*. (2008) found that male *C. latirostris* had total leukocyte, eosinophil, heterophil, and monocytes levels greater than those of females. Similarly, Stacy and Whitaker (2000) found mean WBC count and mean heterophil counts were significantly greater in adult male *C. palustris* than in adult females. Troiano *et al*. (1996) found inter-sex differences too, both in total count and differential leukocyte in other caiman species. Considering the results obtained in this study, sex, unlike age, should not be considered a key variable in studies that may use *S. merianae* as a model. In conclusion, the baseline values obtained in this study contributed to knowledge of IS of the *Salvator merianane*. Thus, of these four immunologic biomarkers studied, three showed differences among age classes, unlike sexes, in the analyzed species. These variations among age classes should be considered in future studies that may use *S. merianae* as sentinel model.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Ávila-Pires, T. (Ed.). 1995. Lizards of *Brazilian Amazonia* (Reptilia: Squamata) Leiden: Zoologische. Verhandelinger, pp: 706-710.

Avrameas, S. 1991. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol. Today 12, 154-159.

Barboza, N., Mussart, N., Coppo, J., Fioranelli, S. and Koza, G. 2008. Internal medium of *Caiman latirostris* in captivity. Influence of sex, growth, and season. Res. Vet. 19, 33-41.

Benatuil, L., Kaye, J., Cretin, N., Godwin, J., Cariappa, A., Pillai, S. and Iacomini, J. 2008. Ig knock in mice producing anti-carbohydrate antibodies: Breakthrough of B-cells producing low affinity anti-self antibodies. J. Immunol. 180, 3839-3848.

Burns, L., Meade, B. and Munson, A. 1996. Toxic Responses of the Immune System. In: Casarett & Doull's Toxicology: Basic Science of Poisons. 5th Edition. (Klaassen C, Ed.). New York: McGraw Hill, pp: 355-402.

Cabagna Zenklusen, M., Lajmanovich, R., Attademo, A., Peltzer, P., Junges, C., Fiorenza Biancucci, G. and Bassó, A. 2011. Hematología y citoquímica de las células sanguíneas de *Rhinella fernandezae* (Anura: Bufonidae) en Espinal y Delta-Islas del río Paraná, Argentina. Rev. Biol. Trop. 59, 17-28.

Charipper, H. 1928. Studies on the Arneth count. - XII.The effect of the injection of thyroid extract on the polynuclear count in a perennibranchiate amphibian (*Necturus maculosus*). Exp. Physiol. 19, 109-113.

Claver, J. and Quaglia, A. 2009. Comparative morphology, development, and function of blood cells in non-mammalian vertebrates. J. Exp. Pet Med. 18, 87-97.

Collado, V., Porras, R., de Simón, M. and Lucía, E. 2008. The innate immune system: Mechanisms. Rev. Comp. Vet. 2, 1-16.

de Castro, E. and Galetti, M. 2004. Numbers of blood cells and their variation. In: Biology of Reptiles (Gans C, and Parsons T, Eds.). New York: Academic Press, pp: 93-109.

Embert, D., Fitzgerald, L. and Waldez, F. 2009. *Tupinambis merianae*. In: IUCN 2010. IUCN Red List of Threatened Species; Version 2010.4. [Accessed: May 2015]. At: www.iucnredlist.org.

García, B., Rubio, F. and Carrasco, M. 1997. Hematología. Citología, fisiología y Patología de hematíes y leucocitos. Paraninfo, Madrid, España.

Gibbons, J., Scott, D., Ryan, T., Buhlmann, K., Tuberville, T., Metts, B., Greene, J., Mills, T., Leiden, Y., Poppy, S. and Winne, C.T. 2000. The global decline of reptiles, déjà vu amphibians. Biosci. 50, 653-666.

González Fernández, A. 2003. Phylogeny of the Immune System. In: Immunology Online Chapter 16. Madrid: Universidad de Córdoba & Sweden Diagnostics. [Accessed: April 2016]. At: http:// www.vi.cl/foro/topic/5698-capitulos-de-inmunologa-apuntes/page_st_ 100.

Hall, B. 1978. Note on the husbandry, behavior, and breeding of captive tegu lizards *Tupinambis teguixin*. Intl. Zoo Yearbook 1978, 91-101.

Harvey, M., Ugueto, G. and Gutberlet, R. 2012. Review of Teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). Zootaxa. 34, 1-156.

Hernández Coronado, M. 2007. Evaluación de la Intoxicación Aguda de Atrazina Sobre la Respuesta Inmune de Tilapia (*Oreochromis niloticus*). Centro Universitario de Ciencias Biológicas y Agropecuarias. Universidad de Guadalajara. Doctoral Thesis.

Hoffmann, J., Kafatos, F., Janeway, C. and Ezekowitz, R. 1999. Phylogenetic perspectives in innate immunity. Sci. 284, 1313-1318.

Huber, T. and Zon, L. 1998. Transcriptional regulation of blood formation during Xenopus development. Semin. Immunol. 10, 103-109.

Kanchanapangka, S., Youngprapakorn, P., Pipatpanukul, K., Krobpan, S. and Kongthaworn, N. 1999. Differentiation of crocodilian granulocytes via histochemical techniques. 4th symposium Diseases in Asian Aquaculture, Cebu City. Philippines. #43.

Khan, S., Epstein, J., Olival, K., Hassan, M., Hossain, M., Rahman, K. and Desmond, J. 2016. Hematology and serum chemistry reference values of stray dogs in Bangladesh. Open Vet. J. 1, 13-20.

Latorre, M., López González, E., Larriera, A., Poletta, G. and Siroski, P. 2013. Effects of in vivo exposure to Roundup® on immune system of *Caiman latirostris*. J. Inmunotoxicol. 10, 349-354.

Latorre, M., López González, E., Siroski, P. and Poletta, G. 2015. Basal frequency of micronuclei and hematological parameters in the side-necked turtle, *Phrynops hilarii*. Acta Herpet. 10, 31-37.

Lewis, S., Bates, I. and Bain, B. 2008. Hematología Práctica. 10th Edition. Madrid, Elsevier.

Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N. and Pacheco, M. 2015. Inside the red-box: Applications of hematology in wildlife monitoring and ecosystem health assessment. Sci. Total Environ. 514, 322-332.

Matson, K., Ricklefs, R. and Klasing, K. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Dev. Comp. Immunol. 29, 275-286.

Merchant, M., Pallansch, M., Paulman, R., Wells, J., Nalca, A. and Ptak, R. 2005. Antiviral activity of serum from the American alligator (*Alligator mississippiensis*). Antiviral Res. 66, 35-38.

Merchant, M. and Britton, A. 2006. Characterization of serum complement activity of saltwater (*Crocodylus porosus*) and freshwater (*Crocodylus johnstoni*) crocodiles. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 143, 488-493.

Modesto, K. and Martinez, C. 2010. Effects of Roundup Transorb on fish: Hematology, anti-oxidant defenses and acetylcholinesterase activity. Chemosphere 81, 781-787.

Montali, R. 1988. Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). J. Comp. Pathol. 99, 1-20.

Mussart, N., Barboza, N., Fioranelli, S., Koza, G., Prado, W. and Coppo, J. 2006. Age, sex, year season, and handling system modify the leukocytal parameters from captive *Caiman latirostris* and *Caiman yacare* (Crocodylia: Alligatoridae). Res. Vet. 17, 3-10.

Novoa-Fajardo, D., Benítez-Tumay, I., Corredor-Matus, J. and Rodríguez-Pulido, J. 2008. Hallazgos hematológicos en iguana verde suramericana (*Iguana iguana*), de ejemplares ubicados en zona urbana y suburbana de Villavicencio (Meta). Orinoquia. 12, 67-79.

NSTRC (National Scientific and Technical Research Council). 2005. Reference Ethical Framework for Biomedics Research: Ethical Principles for Research with Laboratory, Farm, and Wild Animals, #1047. Anexo II, Buenos Aires, Argentina: CONICET.

Olson, G.A., Hessler, J.R and Faith, R.E. 1977. Techniques for the blood collection and intravascular infusion of reptiles. Lab. Anim. Sci. 25, 783-786.

Parmentier, H., Lammers, A., Hoekman, J., de Vries Reilingh, G., Zaanen, I. and Savelkoul, H. 2004. Different levels of natural antibodies in chickens divergently selected for specific antibody responses. Dev. Comp. Immunol. 28, 39-49.

Ray, S., Mukherjee, S., Bhunia, N., Bhunia, A. and Ray, M. 2015. Immunotoxicological threats of pollutants in aquatic invertebrates. Emerging pollutants in the environment -Current and further implications. InTech. 6, 149-167.

Rios, C., Zapata, B., Marín, M. P., Pacheco, S., Rivera, K., González, B.A. and Bas, F. 2003. Cambios hematológicos, bioquímica sanguínea y cortisol sérico en crías de guanaco (*lama guanicoe*) en cautiverio desde el nacimiento al destete. Av. Cienc. Vet. 18, 1-2.

Salakij, C., Salakij, J., Apibal, S., Narkkong, N., Chanhome, L. and Rochanapat, N. 2002. Hematology, morphology, cytochemical staining, and ultrastructural characteristics of blood cells in king cobras (*Ophiophagus hannah*). Vet. Clin. Pathol. 31, 116-126.

Salinas, Z., Salas, N., Baraquet, M. and Martino, A. 2015. Biomarcadores hematológicos del sapo común *Bufo arenarum* en ecosistemas alterados de la provincia de Córdoba. Acta toxicol. Argent. 23, 25-35.

Santos, X. and Llorente, G. 2009. Decline of a common reptile: Case study of the viperine snake *Natrix maura* in a Mediterranean wetland. Acta Herpet. 4(2), 161-169.

Schaumburg, L., Poletta, G., Siroski, P. and Mudry, M. 2012. Baseline values of micronuclei and Comet assay in lizard *Tupinambis merianae* (Teiidae, Squamata). Ecotoxicol. Environ. Saf. 84, 99-103.

Schaumburg, L., Siroski, P., Poletta, G. and Mudry, M. 2016. Genotoxicity induced by Roundup® (Glyphosate) in tegu lizard (*Salvator merianae*) embryos. Pesticide Biochem. Physiol. 130, 71-78.

Silvestre, A. 2014. Hematología y bioquímica en reptiles. EN PORTADA. Barcelona–Espanha, pp: 32-35.

Siroski, P. 2011. Caracterización del Sistema de Complemento e Identificación de Componentes del Sistema Inmune Innato del Yacaré Overo (*Caiman latirostris*). Doctoral Thesis.

Siroski, P., Merchant, M., Parachu Marco, V., Piña, C. and Ortega, H. 2010. Characterization of serum complement activity of broad snouted caiman (*Caiman latirostris*, Crocodilia: Alligatori-dae). Zool. Stud. 49, 64-70.

Siroski, P., Poletta, G., Latorre, M., Merchant, M., Ortega, H. and Mudry, M. 2016. Immunotoxicity of commercial-mixed glyphosate in broad snouted caiman (*Caiman latirostris*). Chem-Biol. Interact. 244, 64-70.

Sparkman, A. and Palacios, M. 2009. A test of life-history theories of immune defense in two ecotypes of the garter snake, *Thamnophis elegans*. J. Anim. Ecol. 78, 1242-1248.

Stacy, B. and Whitaker, N. 2000. Hematology and blood biochemistry of captive mugger crocodiles (*Crocodylus palustris*). J. Zoo Wild. Med. 31, 339-347.

Stacy, N., Alleman, A. and Sayler, K. 2011. Diagnostic hematology of reptiles. Clin. Lab. Med. 1, 87-108.

Strasser, A., Teltscher, A., May, B., Sanders, C. and Niedermüller, H. 2000. Age-associated changes in the immune system of German shepherd dogs. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 47(3), 181-192.

Troiano, J., Silva, M., Esarte, M., Márquez, A. and Mira, G. 1996. Valores hematológicos de las especies argentinas del genero Caiman (Crocodylia-Alligatoridae). Rev. Facena. 12, 111-117.

Troiano, J. and Silva, M. 1998. Valores hematológicos de referencia en tortuga terrestre argentina (*Chelonoidis chilensis chilensis*). Anal Vet. 18, 47-51.

Ujvari, B. and Madsen, T. 2011. Do natural antibodies compensate for humoral immuno-senescence in tropical pythons? Func. Ecol. 25, 813-817.

Weir, S., Yu, S., Talent, L., Maul, J., Anderson, T. and Salice, C. 2015. Improving reptile ecological risk assessment: oral and dermal toxicity of pesticides to a common lizard species (*Sceloporus occidentalis*). Environ. Toxicol. Chem. 34, 1778-1786.

Winck, G., Blanco, C. and Cechin, S. 2011. Population ecology of *Tupinambis merianae* (Squamata, Teiidae): Home-range, activity and space use. Anim. Biol. 61, 493-510.

Yanosky, A. and Mercolli, C. 1992. Tegu lizard (*Tupinambis teguixin*) management in captivity at El Bagual Ecological Reserve. Argentina Arch. Zootec. 41, 41-51.

Zimmerman, L., Vogel, L. and Bowden, R. 2010. Understanding the vertebrate immune system: Insights from the reptilian perspective. J. Exp. Biol. 213, 661-671.

Zimmerman, L., Clairardin, S., Paitz, R., Hicke, J., LaMagdeleine, K., Vogel, L. and Bowden, R. 2013. Humoral immune responses are maintained with age in a long-lived ectotherm, the red-eared slider turtle. J. Exp. Biol. 216, 633-640.