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## Hematological Parameters of Geese Used in Biomedical Research

### ABSTRACT

Domestic geese are birds of zootechnical interest commonly created for ornamentation and guard in farms but are also useful for biomedical research, once they supply blood as a byproduct for laboratory analysis. The study aimed to contribute to the completion of health data available on these animals to trace a hematological profile of domestic geese that supply blood for research and provide data on the influence of periodic collections to the health of these animals. Ten Chinese geese (*Anser domesticus*), white and males, were kept in a research center installation. Four blood samples were performed weekly after the 1st collection, the sample with greater volume was sent to the laboratories of the Evandro Chagas Institute to be used in the arbovirus tests. The hematological evaluations observed values of packed cell volume (PCV), total number of erythrocytes (Hm), total number of leukocytes (Lc) and differential leukocyte count and the number of thrombocytes (Tb). All the animals were weighed and correlation of volume of blood collected from the animal's weight was performed. No differences were found among the means obtained in the hematological values of the 1st collection and the subsequent collections demonstrating that the periodic collection in geese, when performed in obedience to the correlation between animal's weight and blood volume, does not cause significant alterations in the animal's hematological profile. The results of the hematological profile obtained in this study will add to the biological data of species available allowing a better health assessment of these animals in the creation of environments and in animal research facilities.

### INTRODUCTION

The periodic blood collection in laboratory animals is performed in experimental procedures, for the determination of biochemical and hematological values and other analyzes, and also to serve as supply to laboratories as raw material for the manufacture of laboratory tests. The volume and frequency of blood collection in an animal should receive special attention in relation to the effects that the frequent collection can cause, because the well-being may be adversely affected and may still be variability in hematological parameters caused by possible immunosuppression, decreased cardiac output, decrease in blood pressure, anemia, hemorrhagic shock and leading to mortality (Morton *et al.*, 1993; Raabe *et al.*, 2011).

The existing *Guidelines* for laboratory animals cite the maximum volume and frequency of blood to be collected for species of mammals, especially rodents. Even so, the publications disagree in relation to the maximum volume to be collected and the ideal collection intervals for the animals recovery. Diehl and collaborators (2001) mention that, in



mice, only 7.5% of the total volume of blood can be collected on a weekly basis, 10% every two weeks and 15% every 4 weeks for a same individual, while Morton and collaborators (1993) recommend that 10% of the total volume of blood can be collected every 3 or 4 weeks for the individual's full recovery and only 1%, in case daily collection is needed. An experiment conducted in mice, during 6 consecutive weeks, showed that the weekly collection of up to 25% of the total volume of blood does not cause hematological alterations in animals, with rapid recovery, after one week, of the normal values for individuals (Raabe *et al.*, 2011).

Regarding the birds, we only know that clinically healthy birds have total blood volume of approximately 6 to 11 mL/100g PV and that the maximum amount to be collected in a single collection, is up to 10% PV (Samour, 2005). However, there is no publication that refers to the interval among the blood samples of the maximum volume of blood collected in birds.

Geese are not usually cited as laboratory animals, even being blood supplier birds as a byproduct for laboratory analysis. This is because they are birds commonly used with the purpose of ornamentation and guard in farms. The goose blood is used for immunological tests as the hemagglutination and hemagglutination inhibition of virus from birds and mammals (Sexton *et al.*, 1994; Davis, 2003; Kurskaia *et al.*, 2009) and, for this reason, it is one of the species created by the Section of Creation and Production of Laboratory Animals (SACPA) of the Evandro Chagas Institute (IEC) aiming to meet weekly demand for the use of the blood of these animals in laboratories that are references of arboviruses.

The hematological values of domestic geese (*Anser domesticus*) available in the literature do not have the full values of parameters of red and white series, therefore they do not help totally the assessment of the health state of geese kept in commercial creations nor those kept in installations for scientific use and used in biomedical research.

The main cell types found in the blood of the birds are: erythrocytes, thrombocytes and leukocytes. These latter are constituted by granulocytes (heterophile, eosinophils and basophils), lymphocytes and monocytes (Campbell, 1994).

It is known that the erythrocytes (red blood cells) or birds are nucleated and larger than cells of mammals. The heterophiles have similar function to the neutrophils in mammals and are the main phagocytic cells involved in the inflammatory response

(Capitelli & Crosta, 2013), besides being considered stress markers in some studies with birds, and there is also a correlation value between lymphocytes and heterophile known as an indicator of chronic stress in birds (Leclerc *et al.*, 2017; Ludwig *et al.*, 2017). Whereas the thrombocytes have functions related to hemostasis and the thromboplastin production, but also perform phagocytic function. The count of thrombocytes is normally not performed in the bird's clinical routine, because the formation of aggregates is frequent, making it difficult to count, in addition to their identification being compromised, and because the proportion between the nucleus and the cytoplasm is high, it is difficult to differentiate them from the smaller leukocytes. Thrombocytopenia may be present in cases of erythrocytes destruction, as in septicemia or disseminated intravascular coagulation, or even collection of excess volumes (Mitchell & Johns, 2008).

To perform hematological analyzes in birds, it is common to use automated hematology apparatus, routinely used in veterinary laboratories, because these pieces of equipment use the impedance, which measures the number and the electrical properties of the particles that pass through some opening, which becomes inefficient in the presence of nucleus in the erythrocytes, since these will interfere with the leukocyte count (Capitelli & Crosta, 2013). Thus, direct and indirect manual methods have been developed and used for hematological analyzes in birds (Bahense, 2010).

Thus, the study sought to use manual methods recommended for birds hematological analysis aiming to draw a hematological profile of domestic geese that supply blood for research and are kept in SACPA/IEC, noting also the occurrence of possible changes in these parameters resulting from the periodic blood collection.

## **MATERIAL AND METHOD**

### **Ethical aspects and location**

Upon approval of CEUA/IEC number 15/2016, 10 geese specimens were used (*Anser domesticus*) of breed Swan/Chinese, white, males, adult with ages ranging from 3 to 5 years, weighing between 3 to 6.2 kg, belonging to the squad of Section of Creation and Production of Laboratory Animals (SACPA) of Evandro Chagas Institute (IEC/SVS/MS), located in Ananindeua-Pará (1°22'34"S, 48°22'54"W), which were used for the blood supply to the laboratory of arboviruses and



which had not been used for blood collection in a minimum period of 45 days before the beginning of this work.

### **Experimental management and parameters evaluated**

The animals were kept in an open environment with greenhouse having access to a swimming pool and were fed daily with specific ration for geese (Megazoo®) and water *ad libitum*. The blood collections were carried out between the months of April and July.

5 blood samples were collected from each individual divided in:

1st Collection - Day 0. 8.5 mL of blood were collected from each animal, being 8 mL sent to the laboratory of arboviruses and 0.5 mL for hematologic evaluation. This collection intended to demonstrate the animals normal hematological parameters.

2nd Collection - 8 days after the 1st collection

3rd Collection - 15 days after the 1st collection

4th Collection - 22 days after the 1st collection

5th Collection - 29 days after the 1st collection

The samples were collected, after physical restraint, through venipuncture in the left brachial vein using 22G needle attached to 3-mL syringe, and the samples immediately potted in microtainers tubes of 1 mL, and always performed in the morning, until 09:00 pm. All samples were processed in the Laboratory for Quality Control of SACPA, where the preparation of the blood smears, the separation of part of the sample in the capillary tubes and the hematimetric reading in a Neubauer chamber, of each sample, do not have calibration for reading of nucleated blood cells.

For the evaluation of the packed cell volume (PCV) or hematocrit, a part of each blood sample was placed in a hematocrit tube, until approximately 2/3 of its capacity, and centrifugation was processed for 5 minutes at 10,000g. The values were measured by means of a micro-hematocrit ruler, aligning the upper meniscus (plasma) with the top line of the ruler, sliding up to match the lower limit of the column of red corpuscles with the bottom line of the ruler, and the values were expressed as a percentage (%).

The counts of the total number of erythrocytes (Hm), total number of leukocytes (Lc) and

thrombocytes (Tb) were held together, in a same dilution and processed in a Neubauer chamber. After waiting for 1 to 2 minutes for the cells sedimentation, each cell type was counted separately at 40X magnification obeying the technique of inverted "L". The sum obtained was multiplied by the correction factor based on the proportion and volume (height x area) of the dilution that occupies the compartments of the Neubauer chamber. The erythrocytes were counted using 1/5 of the central compartment, and leukocytes, using the four side compartments; the thrombocytes, the whole central compartment. The values obtained for the total number of erythrocytes were expressed in  $\times 10^6/\text{ul}$ , while the values of total number of leukocytes (Lc) and thrombocytes (Tb) expressed in  $\times 10^3/\text{ul}$ .

The leukocytes count was performed by means of microscopy at 100X magnification (immersion) of the smears stained by May-Grünwald Giemsa method (MGG). The leukocytes were observed following the technique of blood smear reading, which uses the middle third of each end side of the stained smear, counting 100 leukocytes in each side, thereby obtaining specific leukocyte counts, in percentage terms, for later calculation of the absolute specific leukocyte expressed in thousands of leukocytes per microliter of blood ( $\times 10^3/\text{ul}$ ).

From the results of the analyzes used in each animal the means and standard deviations were obtained and, from these results, the ANOVA statistical test was applied to assess the significance of the alterations found in individuals, by observing the differences among the values obtained post-collection to supply to the Laboratory of arboviruses, considering  $p \leq 0.05$  (bilateral).

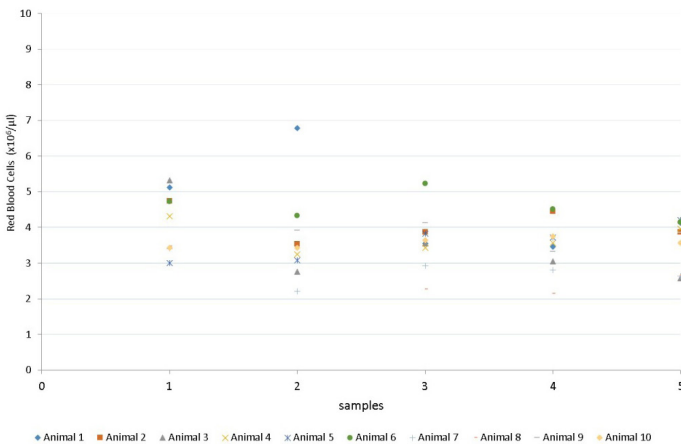
## **RESULTS AND DISCUSSION**

The means obtained for hematological parameters of the geese are shown in Table 1.

There was no statistically significant difference among the means of the studied parameters obtained in the 1st collection (Normal hematological parameters) and after the subsequent collections. Only 2 animals of the study presented values of cells with large variation among the collections (Figure 1).

**Table 1** – Hematological Reference Values for *Anser domesticus* kept in IEC/SVS/MS.

PCV (%)	RBC ( $\times 10^9/\text{ul}$ )	WBC ( $\times 10^3/\text{ul}$ )	Thromb ( $\times 10^3/\text{ul}$ )	Heter ( $\times 10^3/\text{ul}$ )	Bas ( $\times 10^3/\text{ul}$ )	Eos ( $\times 10^3/\text{ul}$ )	Mon ( $\times 10^3/\text{ul}$ )	Lym ( $\times 10^3/\text{ul}$ )
35.7 ( $\pm 5.23$ )	4.091 ( $\pm 0.80$ )	2120 ( $\pm 316.3$ )	52.1 ( $\pm 13.02$ )	22.7 ( $\pm 13.81$ )	22.6 ( $\pm 8.11$ )	34.9 ( $\pm 19.74$ )	1.7 ( $\pm 1.26$ )	18.1 ( $\pm 9.64$ )



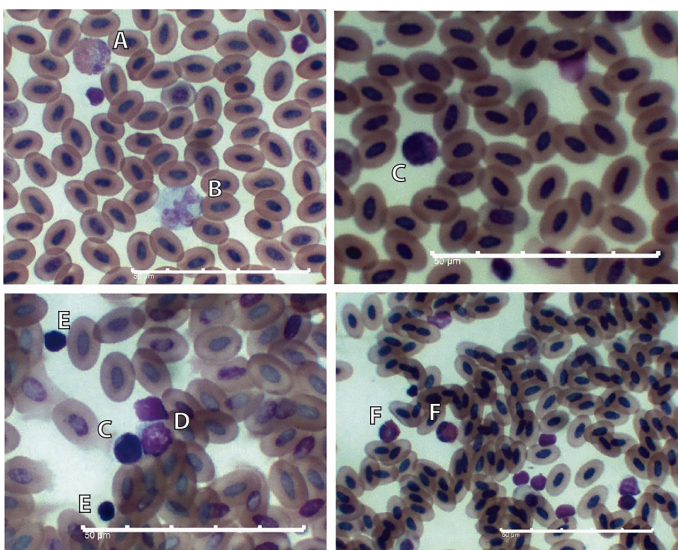
**Figure 1**

The correlation of the animal live weight and volume collected per animal obeyed to the maximum volumes suggested for collection in animals already established. This correlation is shown in Table 2.

**Table 2 – Correlation of Geese Weight and Blood Volume per animal withdrawn for Arbovirus Dept/IEC/SVS/MS.**

Geese	Weight (kg)	Weight/Blood collected (%)
1	4.2	0.19%
2	6.2	0.12%
3	4.3	0.18%
4	4.7	0.17%
5	4.1	0.19%
6	3	0.26%
7	3.7	0.21%
8	5.6	0.14%
9	5.4	0.14%
10	3.9	0.2%

All the cell types were identified in the sample's analysis (Figure 2).



**Figure 2**

The collection of a blood sample for quality is one of the most important parts of hematology, where the

collection technique and care in the procedure directly influences the results obtained (Clark *et al.*, 2009).

As a rule, for most species, it is considered safe to collect up to 10% of the volume of blood in a single collection, which is roughly equivalent to 1% of the animal's body weight (Campbell & Ellis, 2007), with recovery of normal hematological values in 3 to 4 weeks (Feijó *et al.*, 2010). In birds, although the spleen does not function as a reservoir of erythrocytes, in general, there is rapid recovery after blood collection (Clark *et al.*, 2009). This can be explained by the fact that the average life of bird's erythrocytes is shorter than in mammals, thus presenting more rapid regeneration (Capitelli & Crosta, 2013). In this study, the volume of blood collected (0.1 - 0.2% of the body weight of the geese used) complied with the maximum limits of volume per animal and the hematological values obtained for the samples related to subsequent collections demonstrated that there was a speedy recovery expected of the parameters considered normal for each animal.

There are no complete hematological parameters available in the literature for *Anser domesticus*. The hematological values for the species published by Gee and collaborators (1981) and by Ritchie, Harrison & Harrison (1994) highlight only the values for red blood cells, packed cell volume and hemoglobin. The present study contributes to the knowledge of the mean values of other hematological parameters as important as those already available.

According to Campbell (1984), the majority of the species present values for hematocrit between 35% to 55%. Thus, the values found in the geese studied are within the expected values and remain even after the periodic blood collection.

The values for red blood cells of the geese used in the study disagree with the values found in the literature for the species ( $2.6 \times 10^6 / \text{mm}^3$ ) (Gee *et al* 1981; Ritchie *et al*, 1994). Only the values for Packed cell volume corroborate with the findings of the cited authors.

Capitelli & Crosta (2013) state that the predominant leukocyte in most species of birds is the heterophile, there are exceptions, with some species presenting lymphocytic predominance in relation to other leukocyte types. The geese in the study showed a predominance of eosinophils in average values observed before the collection (34.9%), in relation to heterophiles (22.7%) and other lineages.

Although the eosinophilia is rare in birds and the role of the eosinophils in birds is not yet fully clarified





(Mitchell & Johns, 2008), it is not possible to say that the predominance of eosinophils in geese is a pathological alteration, because there are no parameters for this species published. Studies already performed indicate that the eosinophilia in birds has a correlation with chronic parasitism (Schmidt *et al.*, 2007). The parasitism in the studied animals cannot be discarded, since, although these are dewormed semiannually, the environment in which these were kept was open and difficult to control the nematodes reinfestation.

Values published for the *Anser domesticus* kept in captivity in India showed high values of  $89.27 \pm 1.36$  heterophile () in relation to the other leukocytes ( $7.77 \pm 1.30$  for lymphocytes  $2.95 \pm 0.45$ , for monocytes and  $0.0\%$  for basophils) (Hamadami *et al.*, 2014). Whereas in study with the species geese *Anser anser* the prevalence of lymphocytes was noted ( $57.90 \pm 2.56$ ), being followed by heterophiles ( $39.00 \pm 2.27$ ), at the expense of other cell types leukocyte antibodies found ( $0.84 \pm 0.52$  for basophils,  $0.97 \pm 0.48\%$  for eosinophils and  $1.23 \pm 0.67$  for monocytes) (Jahantigh *et al.*, 2016).

It is important to emphasize the difficulty of the implementation and interpretation of hemogram in birds, mainly due to the lack of establishing reference values for all the species, with a variation of values as a function of the population, genetic factors, habitat, sex, age, physiological state, seasonality, etc. Although the goose *Anser domesticus* species is considered a domestic animal, it is difficult to obtain physiological data about this species. Especially in relation to the hematological parameters the publications are rare and, in general, incomplete, even this being one of the best ways to analyze the life and health conditions of these animals.

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