Estudos em Patologia Veterinária

Valeska Regina Reque Ruiz
(Organizadora)

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APRESENTAÇÃO

A Patologia Veterinária é uma área da Medicina Veterinária responsável pelo diagnóstico das doenças nos animais domésticos e selvagens, através do exame clínico do animal, dos tecidos e fluidos corporais. É dividida em dois ramos, a patologia da anatomia e a patologia clínica, ambas realizam o diagnóstico de doenças nos animais verificando se há risco para os humanos manusearem, consumirem ou conviverem com estes, sejam eles animais produtores de alimentos, animais selvagens ou exóticos, ou animais de companhia. Além do diagnóstico os veterinários patologistas têm um papel importante na descoberta de novas formas de tratamento, bem como a investigação científica de doenças pré-existentes, ou descobrindo uma nova doença.

Para tanto o conhecimento da fisiologia animal é importante, e desta forma conhecer o que está alterado nos estados patológicos. Já o conhecimento das patologias deve ser constantemente aprofundado, através de estudos, leituras, cursos e especializações. Desta forma a Editora Atena apresenta o livro Estudos em Patologia Veterinária, o qual traz estudos de patologia de cães, gatos, bovinos, equinos, pinguins, lambaris, mamíferos selvagens e coelhos.

Bom estudo!

Valeska Regina Reque Ruiz

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CAPÍTULO 11

VARIATION OF HETEROPHIL/LYMPHOCYTE RATIO IN REHABILITATION OF MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*, FOSTER 1781)

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ABSTRACT: The heterophil/lymphocyte ratio (H/L) has been proposed as a sensible chronic stress index in birds. This study aims at evaluating the H/L rate in Magellanic penguins impacted or not with oil before, during and after the rehabilitation process. Two blood smears were made of each bird, with an average break of 44 days, colored with Giemsa. The data of the first and the second collect were analyzed individually through the Mann-Whitney U test. The variation of the H/L rate between the two collects was analyzed through the t Test. There was no significant variation in the H/L rate between the two groups in the first collect (without oil=1.68±0.49; with oil =1.61±0.30), demonstrating similar level of stress. In the second collect the group without oil presented smaller H/L rate (1.06±0.29), when compared to the impacted group by oil (1.64±0.33), indicating a reduction in the level of stress of the group without oil. During the rehabilitation process, in other words, between the two collects, the average of the H/L rate of the group without oil corrected for 30 days was -0.79, corresponding to a fall in the level of stress of this group. On the other hand, in the group impacted for oil the average rate was 0.14, bigger (p=0.009). It is concluded that the group with oil has presented bigger H/L rate indicating the persistence of a potential situation of intoxications or stress.

KEYWORDS: Heterophil/Lymphocyte Rate, Oil Impact, Stress, Magellanic Penguins

1 I INTRODUCTION

Magellanic penguins (*Spheniscus magellanicus*) are marine birds which inhabit the extreme south of South America. Their

reproduction sites are located in the south coast of Argentina, Chile and Falklands Island (Malvinas). In Brazilian coast they take place from Rio Grande do Sul to Espírito Santo, with occasional records in other States (NACINOVIC, 2005), being the most common penguin species in Brazilian jurisdictional waters (SILVA FILHO; RUOPPOLO, 2007). After reproductive period, from September to March, these birds leave the colonies and migrate along the Atlantic coast of South America, following displacement of schooling fish and ocean currents, returning to the originate colonies in Spring (WILLIAMS, 1995; SICK, 1997).

The migration is a period in which the penguins are subjected to several stress conditions, especially the young ones, ocean pollution, which face their first migration. There are several threatens to the populations of these birds, including climate changes, commercial fishing, privation of nourishment, ocean pollution by oil and garbage, physical damages, human intervention, predators and illnesses (BOERSMA; STOKES, 1995; VLECK et al., 2002; PETRY; FONSECA, 2002; STOKES; BOERSMA, 2014).

Accidental oil spill or the result of cleaning of tanks during maritime transport affects severely the marine birds in South America and in many other locations worldwide (BORBOROGLU et al., 2006; SILVA FILHO; RUOPPOLO, 2007). It is known that marine birds are negatively affected by oil, particularly the birds which remain most of the time on the surface (PIATT et al. 1990; RUPPOLO et al., 2014).

There are increasing evidences that oil spills have resulted in damages to the immune system of wild birds, rehabilitated or not. This could mean that the oil impact in the marine bird populations is bigger, and maybe more lasting than previously thought (BRIGGS et al., 1996).

Some indicators used to measure the stress in free-living birds include the hematocrit (MORTON, 1993), sedimentation rate of red blood cells (SVENSSON; MERILA, 1996), glycosylated hemoglobin (ANDERSSON; GUSTAFSSON, 1995) and the level of corticosterone. Another stress evaluation index in birds is the heterophil/lymphocyte ratio (H/L) in peripheral blood (VLECK et al., 2000; SCOPE et al., 2002).

This relation has been proposed as a chronic stress sensible index in birds (RUIZ et al., 2002; BORGES et al., 2003). The H/L rate reflects the immune state of birds and it is influenced by acute or chronic stress in the individuals (MALLORY et al., 2010).

The evaluation of stress based on the H/L rate presents several advantages about the determination of hormones levels (DAVIS et al., 2008). Heterophils can be high because they participate in fighting the infections and/or lymphocytes can decrease as part of general response to stress. This suggests that the H/L proportion is a response of longer duration to stress than the level of corticosterone (DEIN, 1986).

In the extreme south of Brazil the Magellanic penguins rescued on beaches due to weaknesses, illnesses, lost from the group and oil contamination are led to Marine Animals Recovering Center *"Centro de Recuperação de Animais Marinhos*" (CRAM) located in the Oceanographic Museum "Prof. Eliézer de C. Rios", of Federal University

of Rio Grande, Rio Grande, RS, Brazil.

This study aimed at evaluating the heterophil/lymphocyte ratio in Magellanic penguins impacted or not by oil before, during and after the rehabilitation process of these birds.

2 I MATERIALS AND METHODS

It has been studied seventeen young animals captured at the Cassino beach (Rio Grande – RS) in 2006 and 2007, presenting conditions such as dehydration, starvation, oil impact, parasite illnesses, and stress for handling and transport. The penguins were taken to CRAM / FURG – RS to be rehabilitated and released back into the wild. According to the protocol preconized by Ruoppolo et al. (2004), it has been carried out periodical blood collects from medial metatarsal vein with 25x7 needles for follow up general conditions of individuals. The penguins in this study were divided into two groups: the ones without oil (n=11) and the ones with oil (n=6).

It has been performed two blood smears of each bird, with 20 to 113 days break (44 days break), dry in room temperature for 24 hours and fixed in methanol for 15 minutes. The material was taken to the UFPEL genetics laboratory and colored with Giemsa 5% for 20 minutes (MARTINS, 2006).

It has been counted 5.000 blood cells per individual, in ordinary, binocular, optical microscope with 100X objective and oculars of 10X, differentiating heterophils from lymphocytes observed, for the calculation of the proportion heterophil/lymphocyte, according to methodology described by Ruiz et al. (2002). The data from the first and the second collecting were analyzed individually through the Mann-Whitney U test. For analysis of the variation of the H/L rate between the two collects it was elaborated a rate using subtraction value between the second and first collect, adjusting for a period of 30 days. This analysis was carried out through the t Test.

3 I RESULTS AND DISCUSSION

The measures and standard deviation of the H/L rate for the group with oil and without oil in both collects and the value of p for analysis of each collect are described in Table 1.

Collects	H/L rate *		p
	Without oil	with oil	
Collect 1	1.68±0.49	1.61±0.30	0.9199
Collect 2	1.06±0.29	1.64±0.33	0.0067

Table 1. Average values of heterophils and lymphocytes count, and from the H/L rate of Magellanic penguins in both blood sample collects, in the extreme south of Rio Grande do Sul.

* Values expressed as average ± standard deviation. Collects analyzed separately.

In the first collect the H/L rate was statiscally equal (p=0.9199) for both groups, varying from 1.61±0.30 to 1.68±0.49. This result shows that in the beginning of the recovering period, both groups have presented similar stress levels. In the second collect the group without oil (1.06±0.29) has presented lower H/L rate (p=0.0067), when compared to the group impacted by oil (1.64±0.33), indicating a reduction of the stress level of the group without oil.

During the rehabilitation process, in other words, between the two collects, the H/L average of the group without oil corrected for 30 days was -0.782627, corresponding to a drop in the stress level of this group. On the other hand, in the impacted group by oil the average rate was 0.140400, bigger (p=0.008973) than the previous group. Individually, the birds differ in a significant way in the corticosterone levels and in the H/L rate. Thus, it is necessary to obtain repeated measures of the same individual before and after the stressing factor, or big samples capable enough to detect the effects of the stressing factor in the population (VLECK et al., 2002).

Similar results to this study were described by Hawkey et al. (1985) in a study with gentoo penguins (*Pygoscelis papua*) in captivity, in which the H/L rate in animals clinically healthy was smaller (H/L=2.3) than in those which presented "bumblefoot"(H/L=4.4), a common condition in birds in captivity involving injuries, inflammation and infection in the digital pads. Heterophils and lymphocytes are the majority of current defense cells in birds and the proportion Heterophils/ lymphocytes is especially sensible to any natural stress factor (DAVIS et al., 2008). Variations in the number of current lymphocytes can occur due to physiological or pathological factors (JAIN, 1986).

In two species of gulls the leucocyte count was different between normal and sick individuals. The H/L rate was around 0.6 in normal individuals, while in the impacted birds by oil, emaciation, infested with parasites or injured this relation increased to 2.9 (AVERBECK, 1992). Some alterations, such as leukocytosis with heterophilia may occur in situations of intoxication or stress (CAMPBELL, 2007).

Coraiola (2012), who determined the H/L rate in young and adults Magellanic penguins, weaken, injured, and/or impacted by oil, in rehabilitation in the coast of Paraná, Brazil, found bigger H/L in animals which died during the process (3.87±0.57) than in the survivors (2.2±0.3). In these animals the corporal score has presented positive correlation with hematocrit and Total Plasmatic Proteins, and negative correlation with the H/L rate. The same way, there was no significant difference in the H/L rate of penguins rehabilitated to first collect (2.2±0.3) in relation to the second (1.74±0.26), similar result to the one found in this study.

Sopezki et al. (2007), who researched young and adult Magellanic penguins, weaken, injured, and/or impacted by oil, in rehabilitation in Rio Grande do Sul, did not find significant difference in the H/L rate before (1.71 ± 0.46) and after (0.99 ± 0.29) the rehabilitation, nevertheless, the analysis did not consider the groups without oil and oil separately.

4 I CONCLUSION

It is concluded that, there was no difference in the H/L rate in the beginning of the rehabilitation process of penguins in the groups with oil and without oil, nevertheless, in a second moment the individuals with oil have presented bigger H/L relation, indicating the persistence of a situational potential of intoxications or stress.

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