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Dynamics of health of wild capybaras: biochemical and physiological parameters

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Abstract: Assessments of generic indices of health in wildlife populations increase our knowledge about the natural history of animal species and provide useful information for ecoepidemiological studies. However, there have been minimal studies on the parameters of hematological and biochemical parameters in wildlife. The goal of this study was to evaluate physiological dynamics of wild capybaras (*Hydrochoerus hydrochaeris*). To this aim, we evaluated biochemical and physiological parameters (blood cell counts, serum protein fractions, and spleen mass) and assessed their variability with sex, body condition, pregnancy status, and season. Samples (n=67) were obtained from a managed population of free-ranging capybaras from Esteros del Iberá (Northeastern Argentina). The main findings reported were i) Kurloff cells (KC) were found in peripheral blood of both sexes, but levels were higher in females; ii) KC, eosinophil (E), and basophil (B) counts were positively associated with body condition; iii) pregnant females had different values of KC, B, and spleen mass than nonpregnant females; iv) albumin and KC (in females) and E and neutrophil (N) counts in males showed a seasonal pattern; and v) protein fractions of capybaras are reported for the first time. Life history traits such as pregnancy, seasonal processes, nutritional status, are reflected in some of the biochemical and physiological parameters evaluated here.

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Introduction

It has long been recognized that life history traits are not only genotype specific but fundamentally environmentally driven (Pigliucci et al. 2006). Wild vertebrates have heterogeneous life trajectories, which include exposure to multiple stressors and infections. The need to adapt to different circumstances requires physiological plasticity. Therefore, investigating physiological parameters can provide valuable information about the life history of wildlife species.

Of the extensive array of physiological variables, the evaluation of hematological and selected biochemical parameters have been extensively used in human and veterinary medicine for monitoring health status and diagnosis (Aleuy et al. 2013, Boes 2010). It has been shown that hematology may be a useful tool to evaluate dynamics of health in wild animals (Beldomenico et al. 2008a). Variations in concentrations of various types of blood cells in an individual, as well as changes in their morphology, are indicative of particular physiological or pathological status. For example, red blood cells (RBCs) and lymphocytes (Ls) may be important indicators of condition and fitness. Nevertheless, biochemical and physiological parameters in wildlife could change in response to different factors (e.g. seasonal changes, competition, population density, resource availability, etc.), and therefore, its interpretation requires caution. For example, low levels of red blood cells are indicative of poor aerobic capacity and result mainly from deficient nourishment and infection or parasitism (Beldomenico et al. 2008a). However, many other disorders (for example: neoplasia, renal disease, toxic compounds) can also cause a decrease in red blood cells (Desnoyers 2010, Fry 2010), though these disorders have seldom or never been documented in wild mammals. Given the dynamic nature of physiological parameters in wildlife, it is useful that the ecological and environmental context of the individuals is taken into account and that a suite of different indexes are employed in combination to evaluate health status in wildlife.

An emblematic Neotropical species is the capybara, *Hydrochoerus hydrochaeris* Linnaeus (Rodentia; Caviidae), the largest living rodent on earth. It is one of the most intensely used wildlife species in South America due to the value of its hide and as a source of protein (Bolkovic et al. 2006). Our knowledge about physiological parameters of this species originates primarily from studies conducted in animals kept in captivity for commercial purposes (Arouca et al. 2000, Corredor-Matus and Rodríguez-Pulido 2010, Muñoz and Montoya 2001, Van der Heijden et al. 2003). Few studies have reported hematological and biochemical parameters in capybara (Corriale et al. 2013, Gioia-Di Chiacchio et al. 2014, Madella et al. 2006).

Capybaras, as other caviomorph rodents, have some unique hematological characteristics. Contrary to what is observed in other mammals, chronic stress induces an increase in circulating levels of eosinophils (Eberhardt et al. 2013), neutrophils (N) have a lobulated nucleus and are called pseudo-eosinophils or heterophils, due to their cytoplasmatic eosinophilic granules (Hawkey and Dennet 1989, Jain 1993). A cell type unique to caviomorphs is the Kurloff cell (KC) (Jara et al. 2005), which is a circulating mononuclear cell with lymphocyte and monocyte properties (Eremin et al. 1980a). The role of KC is not completely understood. Nevertheless, evidence is accumulating concerning their role in the immunological defense of the guinea pig (*Cavia porcellus*). At least in this species, KCs have natural killer activity (Debout et al. 1984, Eremin et al. 1980b). Moreover, increased numbers of KC appear associated with antileukemic effects *in vivo* (Debout et al. 1995). Only a few studies described the occurrence of KC in capybaras (Hawkey and Dennet 1989, Jara et al. 2005, Messick and Willet 1987). Biochemical parameters such as total protein and protein fractions are also useful tools used in human and veterinary medicine to assess the health of an individual (Weiss and Wardrop 2010). However, it is necessary to establish baseline values for a given species to be able to interpret the information they provide. In capybaras, only a few studies reported the values of albumin and total protein (e.g. Corredor-Matus and Rodríguez-Pulido 2010), and in no case did they described the protein fractions. An alteration of the pattern of these fractions is useful information when interpreted concomitantly with other clinical and laboratory findings (Thomas 2000).

The spleen mass is a useful metric employed to assess immunological investment in commercial birds and laboratory rodents (Hõrak et al. 2006), but it has been little explored in wildlife species because further studies are needed to establish its significance (Vicente et al. 2007).

To contribute to our knowledge of the ecophysiology of capybaras under natural circumstances, here, we report the biochemical and physiological parameters of free-ranging individuals from Esteros del Iberá, Argentina, and assess their variability with sex, body condition, pregnancy status, and season.

Materials and methods

Study area

The ranch "Rincón del Socorro", Corrientes province, Argentina (28°36′ S 57°49′ W), is a 12.000-ha private reserve inserted in the macrosystem Esteros del Ibera. This ecosystem's landscape is dominated by swamps and marshlands connected in an extensive system of shallow lakes (Neiff and Poi de Neiff 2005). The climate is subtropical and humid without a dry season. However, in spring (October–December) and summer (January–March), both rainfall and temperatures are higher than in the rest of the year. The lowest mean monthly minimum temperature is 16–17°C in June and July and highest mean monthly maximum temperature is 27–28°C in January and February (range: -5 to 44°C during the year). The cumulative precipitation recorded by a meteorological station set near the ranch was 1487 mm in 2010, 1280 mm in 2011, and 1426 mm in 2012 (Red Iberá-Entidad Binacional Yacyretá).

Samples

Over a 2-year period (August 2010–September 2012), three capybara per month were euthanized as part of a program to limit overpopulation, authorized by the Dirección de Recursos Naturales of Corrientes Province. Sixty-seven wild capybaras were euthanized by gunshot, and blood samples were taken immediately after death from the vena cava and stored in tubes with and without anticoagulant (5 mM EDTA), which were kept refrigerated (4°C) and processed within 6 h of collection.

Dependent variables

Hematological and biochemical parameters

Samples with anticoagulant were used for complete blood cell counts and blood smears as described in Eberhardt et al. (2013).

Serum samples were used to determine total protein (TP), serum proteinogram, and albumin:globulin ratio (A:G). TP evaluation was carried out by colorimetric assays using an automated biochemical analyzer (Wiener Lab. Group, Argentina).

Serum protein fractions were separated by electrophoresis and quantified in an automated densitometer (Interlab SRL, Italy). From 25 µl of each serum samples, protein fractions were separated by electrophoresis on cellulose acetate strip at pH 8.6 and run using a constant voltage of 260 V for 45 min. After staining, each protein fraction was quantified and the A:G ratio calculated. Elfolab software (Interlab SRL, Italy) was used for data analysis.

Spleen mass

Following dissection, the entire spleen was dissected and weighed using a digital precision scale (Ohaus Traveller).

Independent variables

The biochemical and physiological parameters measured were compared for different sexes, sizes, body conditions, and seasons. Immediately after being euthanized, the animals were weighed (body mass) using a mechanical scale (precision 0.2 kg.), measured (the morphometric measure used in this study was total length, precision 0.5 cm), and then dissected.

The capybara has a gestation of 147–156 days. At 60 days of gestation, capybara embryos are 4–5 cm long and weigh only 7–8 g (López-Barbella 1987). After this, embryos become fetuses and grow exponentially, reaching 15 cm and 250 g at day 90 (Miglino et al. 2013). Considering the above, we divided the pregnancy into two periods: early pregnancy, corresponding to embryos up to 5 cm long, and advanced pregnancy, when embryos were larger. Hence, "pregnancy status" had three levels: nonpregnant, early pregnancy, and advanced pregnancy.

The measure of body condition was a residual index (Green 2001), which was estimated with a linear regression of body mass against total length that included adjustment by pregnancy status (three level factor=nonpregnant–which includes males–early pregnancy, advanced pregnancy). We took into account pregnancy status because in a female with advanced pregnancy, a substantial proportion of its body weight corresponds to the placenta and its contents, which does not directly reflect the body condition of a female.

Owing to the climatic characteristics of the study area (see above), we compared variables between two seasons (spring-summer vs. autumn-winter).

Statistical analysis

Detailed descriptive statistics are offered for each variable. We conducted two types of analysis; first, comparisons between sexes were performed using Mann-Whitney U-tests, and second, within each sex, we assessed the effect of body condition, season, and pregnancy status (the latter only for females), using multivariable linear regressions for normally distributed responses or generalized linear models (GLM) when the response approached the negative binomial distribution. We also included the interaction term season*sampling period (2010–2011 vs. 2011–2012) to verify that any apparent seasonal pattern was consistently found in both study years.

Results

The capybaras were all adults except for one (which was a subadult according to its body mass=27 kg) (Ojasti 2011). Thirty-four were males and 33 were females, and they weighed from 27 to 70 kg (mean=55.3 kg). Their total length ranged from 101 to 146 cm (mean=123.5 cm), and their thoracic circumference was from 65 to 93 cm (mean=84.4 cm). Fifteen of the 33 females were pregnant.

The descriptive statistics of the parameters measured in the free-ranging capybaras sampled are reported in Table 1.

Blood cell morphology

In general, erythrocytes had a center not as pale as those of other mammals (Figure 1A, B). Several blood smears exhibited some red cell morphologic alterations, such as aggregations resembling a pile of coins (rouleaux) and increased variability in its shape (poikilocytosis) (Figure 1D, E).

Lymphocytes were the most frequent WBC in the peripheral blood of all capybaras analyzed, followed by Ns (Table 1). Small and large L were identified; some of them with azurophilic granules in the cytoplasm (Figure 1A, B). N had a condensed, segmented nucleus (with up to five or more segments), and numerous distinctive acidophilic granules dispersed in the cytoplasm (Figure 1C). Eosinophils were distinguished from N due to a less segmented

Table 1: Biochemical and physiological parameters of wild capybaras from Esteros del Iberá, Argentina.

Parameters	Total animals Mean (range)	Sex		Female	
		Male mean (range)	Female mean (range)	Pregnant mean (range)	Nonpregnant mean (range)
Total protein (g/dl)	6.47 (4.26, 7.90)	6.36 (4.26, 7.80)	6.57 (4.70, 7.90)	6.54 (4.70, 7.90)	6.56 (4.95, 7.80)
Albumin (g/dl)	2.96 (2.2, 4.00)	2.88 (2.20, 4.00)	3.04 (2.20, 3.70)	3.07 (2.20, 3.70)	2.97 (2.29, 3.70)
α1-Globulin (g/dl)	0.05 (0.00, 0.10)	0.05 (0.00, 0.10)	0.06 (0.00, 0.10)	0.06 (0.00, 0.10)	0.07 (0.00, 0.10)
α2-Globulin (g/dl)	0.79 (0.25, 1.20)	0.77 (0.30, 1.20)	0.85 (0.25, 1.10)	0.84 (0.50, 1.10)	0.76 (0.25, 1.00)
β-Globulin (g/dl)	1.05 (0.50, 2.00)	1.08 (0.50, 2.00)	1.02 (0.68, 1.60)	1.02 (0.70, 1.50)	0.99 (0.68, 1.60)
γ-Globulin (g/dl)	1.66 (0.80, 3.70)	1.68 (0.90, 3.70)	1.63 (0.80, 3.50)	1.51 (0.95, 2.20)	1.80 (0.80, 3.50)
RBC (millons of cells/μl)	4.92 (1.46, 12.38)	4.65 (1.82, 7.24)	5.24 (1.46, 12.38)	5.01 (3.06, 6.62)	5.51 (1.46, 12.38)
WBC (thousand of cells/μl)	7.81 (1.60, 18.65)	7.12 (1.60, 13.15)	8.60 (2.95, 18.65)	9.06 (3.60, 18.65)	8.07 (2.95, 14.35)
L (thousand of cells/μl)	5.67 (1.26, 15.93)	5.07 (1.26, 10.56)	6.37 (2.12, 15.93)	6.52 (2.21, 15.93)	6.20 (2.13, 10.34)
N (thousand of cells/μl)	1.37 (0.06, 4.60)	1.40 (0.06, 4.60)	1.33 (0.15, 3.07)	1.49 (0.51, 3.07)	1.15 (0.15, 2.66)
E (cells/μl)	375 (15, 1202)	370 (16, 1202)	381 (15, 1160)	418 (113, 1160)	339 (15, 1055)
B (cells/μl)	51 (0, 283)	42 (0, 283)	62 (0, 272)	60 (0, 272)	66 (0, 252)
M (cells/μl)	267 (0, 1165)	210 (0, 1068)	332 (0, 1165)	403 (36, 1165)	250 (0, 971)
KC (cells/μl)	72 (0, 904)	21 (0, 109)[a]	129 (0, 904)[a]	191 (0, 904)[b]	57 (0, 389)[b]
Spleen mass (g)	112.9 (53.3, 164.2)	115.7 (57.1, 164.2)	109.8 (53.3, 161.6)	124.8 (85.6, 124.8)[c]	92.4 (53.3, 161.6)[c]

RBC, red blood cells; WBC, white blood cells; L, lymphocytes; N, neutrophils; E, eosinophils; B, basophils; M, monocytes; KC, Kurloff cells.
[a,b,c]Significant differences between groups.

nucleus, and thick, round, and bright granules that were more acidophilic than Ns and which filled the cytoplasm almost completely (Figure 1D). Monocytes were usually larger than large L with abundant cytoplasm and a pleomorphic nucleus (Figure 1E). Basophils were similar to those of other species, with many round basophilic granules and lobular nucleus (Figure 1F). Kurloff cells, contained a cytoplasmic vacuole (rarely two vacuoles) of variable size, with granular to amorphous material (Figure 1G, H). Their nuclei were pleomorphic, strongly basophilic, surrounded by a scarce slightly basophilic cytoplasm. No immature white cells were found.

Serum proteins

The total protein concentration ranged from 4.26 to 7.90 (g/dl). In all samples, albumin and α (α1-), (α2-), β (β-), and γ (γ-) globulin fractions were identified, although α1-globulin fractions were almost negligible (Figure 2). Albumin was easily identified as a thick band, reflecting its high serum concentration and homogeneous electric charge. It constituted 45.99% (range: 30.30–57.30%) of the total serum protein concentration. The α1-globulin fraction was only 0.80% (range: 0.00–2.20%) of the total serum protein concentration, α2-globulin fraction reached 12.10% (range: 4.00–17.90%), whereas β-globulin was 15.85% (range: 1.80–29.50%) and γ-globulin, 25.03% (range: 12.20–49.20%). The mean A:G ratio was 0.79 (range: 0.48–1.25).

Comparisons: sex, body condition, season, and pregnancy status

Sex

Of all parameters evaluated, the only significant difference between males and females was found for KCs. Females had a higher occurrence (72% in females vs. 45% in males) and higher KC counts (cells/microlitre) than males (p=0.006) (Table 1).

Body condition

Body condition had a significant, generally positive, effect on KC, E, and B counts. There was a strong positive association between KC and body condition in males (Table 2). Females with good body condition had higher E counts than females with decreased body condition (Table 3). Males with good body condition had higher values of B than individuals with low body condition (Tables 2 and 3). Nevertheless, in pregnant females, B counts were negatively associated with body condition, especially during early pregnancy (Figure 3; Table 3).

Season

Independently of pregnancy status, females had KC levels much higher in cold and dry months (autumn-winter;

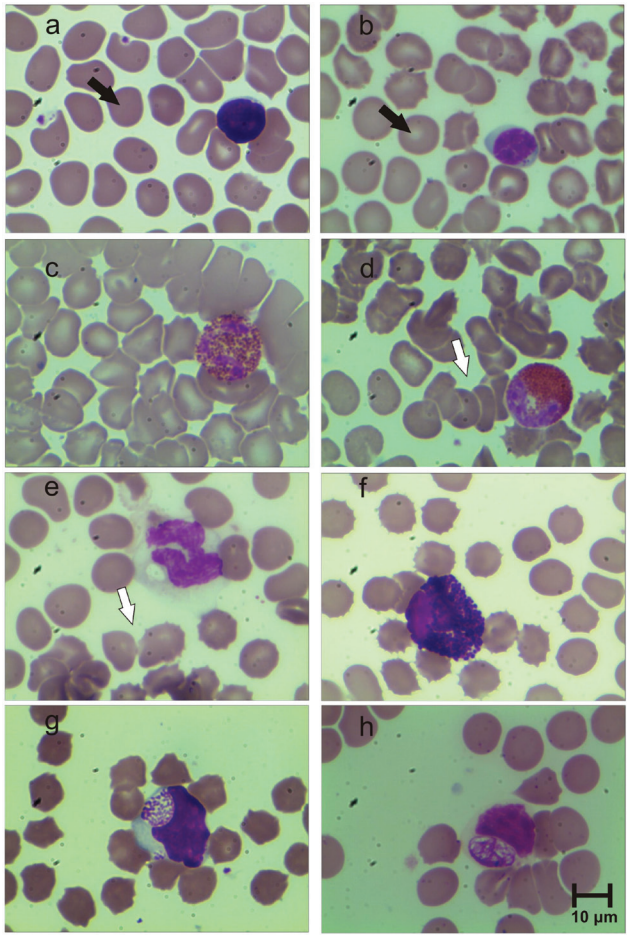

Figure 1: Blood cells in peripheral blood of wildlife capybara. Lymphocytes (a y b); Neutrophil (c); Eosinophil (d); Monocyte (e); Basophil (f) and Kurloff cells (g and h). Black arrows indicates center pale of erythrocytes and white arrows show some red cell morphological alterations (rouleaux and poikilocytosis).

p=0.0307) than in spring-summer (Table 3, Figure 4). In the case of males, the intra-year variation was not consistent between both sampling years (Table 2). In the first period,

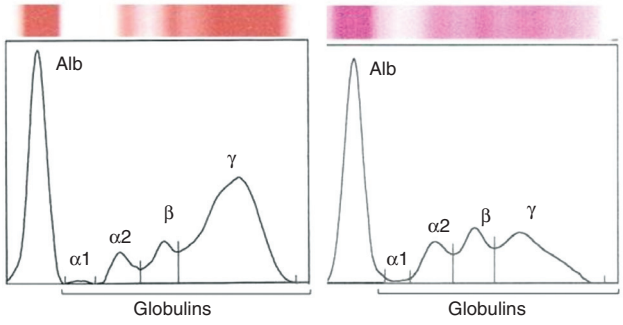

Figure 2: Electrophoretogram of cellulose acetate strip serum protein electrophoresis of serum from wildlife capybara. Left: with medium values of protein fractions; right: with α-globulins value increased.

Table 2: Models describing comparison between biochemical and physiological parameters of wild male capybaras and season, body condition, and period of sampling.

Term	Coefficients	Standard error	p-Value
Model=Kurloff cells~season*period+body condition			
Intercept	2.05787	0.11985	<0.001
$Season_{(autum\text{-}winter)}^{a}$	0.38156	0.13387	0.0044
$Period_{(second)}^{b}$	0.70034	0.11866	<0.001
Body condition	0.26946	0.01774	<0.001
Season*Period	-1.10134	0.17967	<0.001
Model=White blood cells~season*period			
Intercept	5956.3	1062.6	<0.001
$Season_{(autum\text{-}winter)}^{a}$	854.9	1460.5	0.5632
$Period_{(second)}^{b}$	4116.0	1460.5	0.0089
Season*Period^{a,b}	-5807.1	2223.4	0.0145
Model=Lymphocytes~season*period			
Intercept	3842.8	920.4	0.0003
$Season_{(autum\text{-}winter)}^{a}$	1705.5	1265.0	0.1888
$Period_{(second)}^{b}$	2769.0	1265.0	0.0374
Season*Period^{a,b}	-4351.9	1925.7	0.0321
Model=Neutrophils$^{0.3}$~season			
Intercept	9.2532	0.4738	<0.001
$Season_{(autum\text{-}winter)}^{a}$	-2.3160	0.7050	0.00267
Model=Eosinophils$^{0.3}$~season			
Intercept	6.1625	0.3571	<0.001
$Season_{(autum\text{-}winter)}^{a}$	-1.6410	0.5313	0.0044
Model=Monocytes~season*period			
Intercept	4.6590	0.3693	<0.001
$Season_{(autum\text{-}winter)}^{a}$	0.7307	0.5070	0.1495
$Period_{(second)}^{b}$	1.3629	0.5067	0.0072
Season*Period^{a,b}	-3.0257	0.7736	<0.001
Model=Basophils~season*period+body condition			
Intercept	3.640900	0.057619	<0.001
$Season_{(autum\text{-}winter)}^{a}$	0.596715	0.069994	<0.001
Body condition	0.054258	0.008153	<0.001
$Period_{(second)}^{b}$	-0.128061	0.082924	0.123
Season*Period^{a,b}	-1.954435	0.166116	<0.001
Model=Albumin$^{0.2}$~season*period			
Intercept	1.26212	0.01218	<0.001
$Season_{(autum\text{-}winter)}^{a}$	-0.05876	0.01723	0.0019
$Period_{(second)}^{b}$	-0.04072	0.01723	0.0253
Season*Period^{a,b}	0.09065	0.02669	0.0021

GLM with negative binomial response for Kurloff cells, white blood cells, monocytes, and basophiles; LM for lymphocytes, neutrophils, eosinophils, albumin, and spleen mass.

[a]Simple contrasts – reference level: spring-summer (the coefficients reflect comparison with spring-summer).

[b]Simple contrasts – reference level: first period (the coefficients reflect comparison with the first period).

KC counts were higher during autumn-winter and in the second period during spring-summer. The same associations, also in males, were found with B. In the first period, males had higher B during autumn-winter, and in the second period, they had higher levels during spring-summer.

Table 3: Models describing comparison between biochemical and physiological and parameters of wild female capybaras and season, pregnancy status, body condition, and period of sampling.

Term	Coefficients	Standard error	p-Value
Model=Kurloff cells~season+pregnancy			
Intercept	2.94718	0.55001	<0.001
$Season_{(autum\text{-}winter)}^{a}$	1.40900	0.65221	0.0307
$Pregnancy_{(early)}^{b}$	-0.04652	0.81361	0.9544
$Pregnancy_{(advanced)}^{c}$	1.60670	0.72546	0.0268
Model=$Eosinophils^{0.3}$~body condition			
Intercept	5.67499	0.21144	<0.001
Body condition	0.16394	0.04672	0.00159
Model=Basophils~body condition*pregnancy			
Intercept	4.21705	0.37348	<0.001
Body condition	0.10442	0.06851	0.1274
$Pregnancy_{(early)}^{c}$	0.04541	0.66411	0.9455
$Pregnancy_{(advanced)}^{c}$	-1.20539	0.59469	0.0427
Body condition*$pregnancy_{(early)}^{b}$	-0.71507	0.27712	0.0099
Body condition*$pregnancy_{(advanced)}^{b}$	-0.52016	0.13926	<0.001
Model=$Albumin^{0.2}$~season*period			
Intercept	1.26756	0.013583	<0.001
$Season_{(autum\text{-}winter)}^{a}$	-0.04136	0.016072	0.0159
$Period_{(second)}^{c}$	-0.005771	0.018511	0.7576
Season*$period^{a,c}$	0.056084	0.028026	0.0555
Model=Spleen mass~pregnancy			
Intercept	94.900	6.295	<0.001
$Pregnancy_{(early)}^{b}$	24.667	12.054	0.0496
$Pregnancy_{(advanced)}^{b}$	32.807	9.863	0.0023

GLM with negative binomial response for Kurloff cells and basophiles; LM for eosinophils, albumin, and spleen mass.
[a]Simple contrasts – reference level: spring-summer (the coefficients reflect comparison with spring-summer).
[b]Simple contrasts – reference level: nonpregnant (the coefficients reflect comparison with nonpregnant female).
[c]Simple contrasts – reference level: first period (the coefficients reflect comparison with the first period).

In males, E and N showed a seasonal pattern, with higher levels during spring-summer than in autumn-winter (Table 2). Furthermore, males also had higher values of total WBC, L, and M during spring-summer, but this was only observed during the second period of sampling (Table 2).

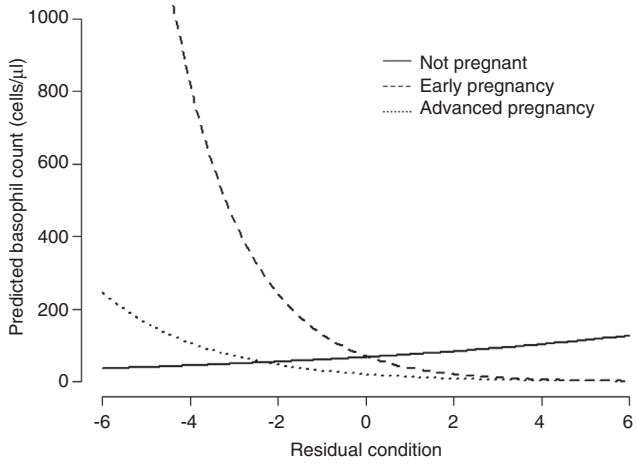

Figure 3: Predicted basophil counts for females by residual body condition. Solid line indicates not pregnant females, dashed line indicates early pregnancy, and dotted line indicates advanced pregnancy females.

Regarding albumin, females had lower levels during autumn-winter than in spring-summer (Table 3). In the case of males, they only had lower albumin levels and A:G ratio during the autumn-winter season in the first sampling period. The trend reversed in the second period (Table 2).

Pregnancy status

Results indicated a significant effect of pregnancy status on KC and B counts and spleen mass. Females with advanced pregnancy had higher KC counts than nonpregnant ones (p=0.0268). KC values were apparently greater in advanced than in early pregnancy, but this difference was not significant (p=0.07). In the case of B, the associations with pregnancy status depended on body condition. Among females in good body condition, basophils were detected only in nonpregnant ones. However, when body

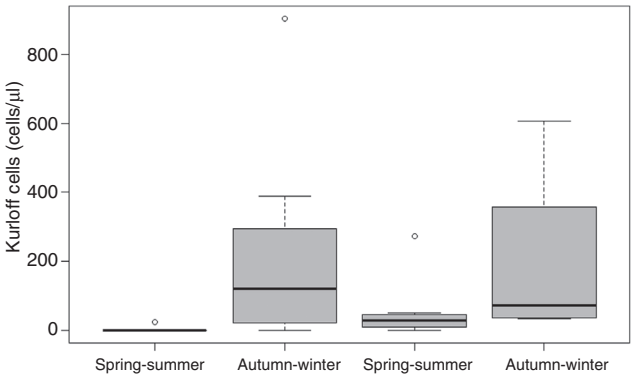

Figure 4: Seasonal pattern of Kurloff cells in females. Boxplots showing the effect of season on Kurloff cells counts in females. Boxplots depict the median (bold bar), 25–75% quartiles (box), 10–90% quantiles (whiskers), and outliers (points).

condition was poor, pregnant females had much higher basophil levels than nonpregnant capybaras, especially during early pregnancy (Table 3; Figure 3).

Pregnant females (early and advanced) had greater splenic mass than nonpregnant ones (p=0.0496 and p=0.0023, respectively; Table 3). No significant difference was observed between early and advanced pregnancies.

Discussion

The main findings reported here were i) KCs were found in peripheral blood of both sexes, but their levels were higher in females; ii) KC, E, and B counts were associated with body condition; iii) pregnant females had different values of KCs, Bs, and splenic mass than nonpregnant females; iv) albumin and KCs (in females) and E and N counts (in males) showed a seasonal pattern; and v) protein fractions of capybaras are reported for the first time.

The blood cell counts reported in this study largely overlap with the ranges reported by other studies carried out in free-ranging and captive capybaras (e.g. Arouca et al. 2000, Corriale et al. 2013).

Capybara erythrocytes showed lack of a discernible pale center in blood cell smears. Other mammals such as rat, mouse (Everds 2007), dog (Harper et al. 2003), marsupial (*Eastern quoll*) (Canfield 1998) show a distinct central pallor. These differences could be due to differences in the degree of biconcavity of red blood cells among species.

In peripheral blood, Ls were the predominant white cells. These results are in agreement with those reported by three previous studies (Corriale et al. 2013, Madella et al. 2006, Van der Heijden et al. 2003). Nevertheless, the three other reports found Ns to be the most frequent circulating leukocyte (Arouca et al. 2000 – only for males – Muñoz and Montoya 2001, Corredor-Matus and Rodríguez-Pulido 2010). The origin of the analyzed animals in the latter studies could explain this discrepancy, as the first three were conducted in wild animals and the remainder three in captivity. Physiological changes induced by stress hormones include increases in numbers of N (neutrophilia) and decreases in L counts (lymphopenia) in blood (Davis et al. 2008). Captivity entails exposure to a number of unnatural stressors, which may influence the N:L relationship (Davis et al. 2008, Vera et al. 2008). The above suggests that capybaras are lymphocytic, like other rodents (Stein et al. 2010, Zimmerman et al. 2010) and that the N:L ratio could be a useful proxy to assess stress in this species. Unexpected findings were those published by Corriale et al. (2013) and Van der Heijden et al. (2003), who found that Es were the second predominant white blood cells. Van der Heijden et al. (2003) only found higher E counts than Ns in tick-infested captive capybaras compared to unifested capybaras. As mentioned above, capybara Ns can be easily confused with Es, as the former also have eosinophilic granules in the cytoplasm (Hawkey and Dennet 1989, Jain 1993). Therefore, it is likely that the very large E counts reported by Corriale et al. (2013), who studied the same population as in this report, may be the result of misclassification of Ns. Our values of E counts were slightly higher than those reported by Arouca et al. (2000) and Corredor-Matus and Rodríguez-Pulido (2010). Eosinophils are involved in the protection against helminth infections (Rothenberg and Hogan 2006). The capybaras sampled in this study were naturally parasitized by the normal parasite community of wild capybaras (data not shown, see Eberhardt 2014), while those of the studies by Arouca et al. (2000) and Corredor-Matus and Rodríguez-Pulido (2010) were done in captive animals, which could explain the higher counts found in this study. In an experiment under controlled conditions, it was shown that capybaras subjected to food restriction and restraint had much poorer body condition than controls, but higher E counts, resulted in lower helminth counts (Eberhardt et al. 2013). Here, however, body condition was negatively associated with E counts, which probably is due to the influence of a myriad of other interacting variables that are present in nature.

Changes in albumin and globulin protein levels can provide early and valuable diagnostic and prognostic information. A change in the A:G ratio is often an indication of a protein dyscrasia; therefore, it is important to establish serum protein reference values and ranges. It is noteworthy that the range for total proteins, albumin, and A:G ratio was in agreement with those reported in

other mammals, including humans (Eckersall 2008, Killingsworth 1979). We identified five fractions of serum proteins in capybaras: albumin and α1-, α2-, β-, and γ- globulin (Figure 2). However, α1-globulin fractions were almost negligible, as observed in sheep (Hailat and Lafl 1998). The pattern of protein fractions of *Hydrochoerus hydrochaeris* was not previously reported. Further studies should be aimed at identifying every component of each fraction.

A key metabolic function of albumin is its role as a transport protein, and it is the main component of blood oncotic pressure. Suboptimal nutrition is reflected on albumin and globulin concentrations (Kaneko 1997). Capybaras are herbivorous semiaquatic mammals that graze near water bodies. They have an optimal consumer foraging, selecting high-quality forage during the season of food abundance and consuming a higher diversity of plants during the season of food scarcity (Barreto and Quintana 2013). Both for females and males (the latter only during the first period), seasonal differences were found in serum albumin levels, with declines in autumn-winter. A reason that could explain this difference is the change in diet during cold and dry months, although decreases in albumin levels may also result from multiple interactions between the capybaras and their environment, for which the determinants of this variability should be further explored.

Regarding KCs, females had significantly higher occurrence and counts than males. Although several studies reported capybara hematological parameters, only Jara et al. (2005) provided some information on KCs. They analyzed blood and several tissues from 22 free-ranging capybaras, only finding KCs in the peripheral blood of a pregnant female. Nevertheless, all individuals presented large numbers of KCs in different tissues. Kurloff cells were originally described for guinea pigs (Revell 1977), in which females also have higher counts than males. Another particular lymphoid cell, the azurocyte, was found in the blood of microtine rodents (voles) (Beldomenico et al. 2009, Mihok et al. 1987). Azurocytes were found in much higher levels in pregnant female voles than in nonpregnant females and males (Beldomenico et al. 2008b). This similar behavior suggests that KCs and azurocytes may be functionally related, carrying out a specific task during pregnancy. It is interesting to note that our results show that KC levels were higher in advanced pregnancy compared to embryonic stage pregnancies, although no significant difference was observed. The same pattern was reported by Mihok et al. (1987) for azurocytes, who found that this cell's levels in blood increased predominantly in the second half of gestation in *Microtus pennsylvanicus*. It is likely that KCs are involved in the normal fetal development via immune defense and participating in mechanisms that protect fetal-placental units, in a similar way to that of uterine NK cells (uNK). In species with hemochorial placentation, uNK cells appear to contribute to the maintenance of decidua that supports placental and fetal development (Bizinotto et al. 2008). In addition, uNK would be involved in spontaneous embryo resorption in rats and mice (e.g. Fonseca et al. 2014). During capybara pregnancy, some of the fetal-placental units undergo spontaneous resorption (16–17%), while the adjacent units remain unaffected (Moreira et al. 2001). It is expected that resorption rates are higher during stressful times. If KCs play a role in resorption, this could explain why female KC levels were much higher in cold and dry months (autumn-winter) than in spring-summer. Beldomenico et al. (2008b) also proposed that azurocytes might be implicated in embryo resorption during times of hardship. Although specific functions of KCs in capybaras should be investigated, our results hint that KCs might play a major role in innate immunity during capybara pregnancy, particularly at the advanced stages.

A related interesting result was the increase in splenic mass during pregnancy, particularly at advanced stages. Jara et al. (2005) demonstrated the presence of KCs in free-ranging capybaras spleen. Ernström and Sandberg (1971) proposed that spleen is the major producer of KCs and that they can be released from this organ into the blood in guinea pig. Our results are in agreement with this notion. The splenic mass may vary due to a lot of factors as parasite infections, sex, age, body condition (Nunn 2002, Vicente et al. 2007), and therefore, its interpretation requires caution. However, some sources of variation in spleen mass do not apply in this case (i.e. slow death) or have been considered in the analysis (i.e. pregnancy).

In males, KCs were positively associated with body condition. Again, this is coincident with the findings of azurocytes in field voles (Beldomenico et al. 2008b). This suggests that in males, KCs may play a role in the protection against infections as their levels could be raised by antigenic stimulation (Revell 1977). Testosterone also could participate in this raise, as KCs are sensitive to sex hormones (Ledingham 1940).

Basophils are precursors of mast cells (MCs), which accompany and deeply affect many steps of reproduction (Garfield et al. 2006). As pregnancy advances, MCs exert an influence on the maintenance of pregnancy (Woidacki et al. 2013). Bytautiene et al. (2008) have showed that uterine MCs increased uterine contractility in pregnant guinea pigs through mediators including histamine and serotonin, uterine responses to these mediators, which

are dependent on gestational age. These mediators could be a stimulus to trigger and/or maintain myometrial contractions during preterm and term labor. If mast cells stimulate myometrial contractions, the fact that we found more Bs (precursors of mast cells) in females in poor condition might suggest that these females could be investing in producing more Bs in order to interrupt reproduction. Reproduction incurs to worthwhile extensive costs that might not be afforded when resources are limited (French et al. 2009). Therefore, the augment of Bs suggests a potential protective mechanism that interrupts gestation when circumstances are unfavorable (i.e. females are in poor body conditions). Embryo resorption may be strategies that females may use to reduce litter size and optimally allocate limited resources (Herrera 1998).

The results reported here shed new light on the physiological dynamics of wild capybaras. Life history traits such as pregnancy, seasonal processes, and nutritional status are reflected in some of the biochemical and physiological parameters evaluated. Further research should increase the study of the patterns presented here and the mechanisms implicated. Linking them to the natural ecological processes of capybaras will enable a better knowledge on the life history of this species.

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