PAPER

Social rank affects the haematologic profile in red deer hinds

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Abstract

We studied the effects of social rank on the haematologic profile in a herd of 24 female Iberian red deer hinds. Social rank hierarchy was determined and blood samples were taken and analysed. After adjusting for age and body mass, dominance ranking showed a significant negative effect (ie, lower values in dominant hinds) on white blood cell (WBC) count, haemoglobin and haematocrit. Our results are similar to those reported for stressed individuals due to physical immobilisation, but do not support the predicted enhanced erythropoiesis due to higher levels of androgens. The results for WBC numbers may also reflect that subordinate hinds must allocate a higher amount of resources to immunity as a result of injuries incurred from dominant hinds, while simultaneously facing restricted access to food sources. For red blood cell (RBC) counts, the results may be due to subordinate hinds likely needing increased haematocrit and haemoglobin levels for fast flight responses. Our data show that social rank influences haematologic profile, and thus it should be considered when correctly interpreting blood analyses in social cervid species.

Introduction

The haematologic profile of cervids is relatively well known. However, comparatively little information is available about how the haematologic profile is influenced by a variety of factors, with age, sex, reproductive status, seasonality and disease being the most important ones proposed.[1] The influence of stress on blood parameters is also well documented, but in cervids it has mainly been studied only in relation to capture, handling and immobilisation procedures. Red blood cell (RBC) count, packed cell volume, haemoglobin (Hgb), white blood cell (WBC) count or lymphocyte count values are significantly higher in excited deer than in resting or chemically restrained ones.[1 2] Thus, the haematologic profiles reported in previous studies on cervids show a wide variability (see Boes[1] for an exhaustive summary), since it is almost impossible to obtain representative resting blood values. According to Wilson and Pauli[3] the excitable nature of deer may be the main reason behind these effects.

However, other stressful situations that may affect the haematologic profile have not been well studied in cervids, such as the stress related to dominance relationships. Is there any physiological mechanism that may suggest an effect of social rank on the haematologic profile? Most social mammals establish hierarchies and an increase in WBC count in submissive animals has been previously reported (Hjarvard and others[4] for pigs). Social rank may also affect the RBC count, as submissive animals are socially stressed[5] and it is well known that stressful situations such as physical immobilisation increases RBC count, haematocrit (Hct) and Hgb.[1] On the other hand, androgen levels and social rank are closely related both in males and females. In female ungulates treatment with androgens consistently raises social rank,[6] and these increased testosterone levels in dominant animals may also have positive effects on the haematologic profile, as it has been observed that testosterone stimulates erythropoiesis in female mice.[7 8] Thus, it can be predicted that social rank influences haematologic profile in several ways: increasing (stress-mediated) WBC counts, and increasing (stress-mediated) or decreasing (androgen-mediated) RBC numbers in submissive hinds. These predictions have not been previously studied in cervids.

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We studied the influence of social rank on the haematologic profile of a captive herd of Iberian red deer hinds (*Cervus elaphus hispanicus*). This is a representative animal model since hinds create stable hierarchies and are subjected to social stress. We carried out our study during lactation. This is the most demanding stage of reproduction[9] and it has been suggested that immune system requirements are covered only after other needs for maintenance, lactation or reproduction have been fulfilled.[10 11] Thus, this competition between lactation and immunity may help to find stronger patterns of variability in the haematologic profile.

Materials and methods

Study animals

This study was carried out on a group of 24 Iberian red deer hinds (*C elaphus hispanicus*) kept at the Experimental Farm of Castilla-La Mancha University in Albacete, Spain (38°57'10''N, 1°47'00''W, 690 m altitude). Hinds were 2–12 years old and individually identified by ear tags and collars. The group was kept in a fenced enclosure of 15 000 m^2, and received ad libitum food based on barley straw and meal from barley, alfalfa, oat and sugar beets (16 per cent crude protein, 11 per cent humidity). Hinds were weighed on blood sampling days, as part of the routine handling activities on the farm.

Blood sampling

Blood samples were obtained from the right jugular vein, always at the same time of day (between one and three hours after dawn) in order to avoid variations due to circadian rhythm[12] while animals were standing inside a small handling box (2m×2 m×0.6 m). No clinical signs of disease were present at the time of the extraction and no sedation was required. Sampling of all animals was performed on six dates during the middle of the lactation period (weekly from July to mid-August). Blood samples for haematology were collected into evacuated tubes containing an anticoagulant (EDTAk3), refrigerated (4°C–8°C) within the first 15 minutes and analysed within two to three hours. Analyses were performed with ADVIA 120 Hematology System (Siemens) at the Complejo Hospitalario Universitario de Albacete, using Advia 120 MultiSpecies System Software. This automated haematology analyser uses photometric measurement for Hgb, optical laser-light scattering for cell enumeration, myeloperoxidase staining coupled with flow cytometry-nuclear globularity analysis for WBC differential, flow cytometry and laser diffraction for RBC and platelet counts.[13] Calibrated commercial controls were run daily before analysing samples. Blood samples were analysed to determine RBC count, Hgb concentration, Hct, mean corpuscular volume (MCV), red cell distribution width (RDW), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), WBC count, lymphocyte count, non-lymphoid leucocyte count, platelet count and mean platelet volume (MPV). As deer lack the myeloperoxidase enzyme,[14] the differentiate count could only classify leucocytes into two types: lymphocytes and non-lymphoid leucocytes. Each datum was the mean of two measures for every sample, with intra-assays coefficients that varied between 0.78 per cent and 2.93 per cent. Mean values after the six sampling days were used for further statistical analyses.

In addition to the herd being well habituated to routine handling,[15] manipulating and sampling procedures were designed to keep stress and health risks at a minimum for the subjects, as according to the European and Spanish laws and current guidelines for ethical use of animals in research.[16]

Dominance index

Interactions to establish social rank were monitored throughout the same period as the blood sampling. Although social hierarchy is considered to be linear and highly stable in red deer hinds,[17] small variations may occur due to changes in weight, body condition or injuries. Thus, the observation period was extended for a few weeks to obtain an adequate amount of data during several days; but not for longer, in order to avoid variations in the hierarchy during the period of blood collection. Fourteen hours of observation were carried out in two-hour periods when there was higher social activity.[17] All interactions were recorded avoiding interferences in the behaviour of the animals, according to the focal group sampling method.[18] The observer remained hidden from the animals outside the enclosure, with optimal observation conditions. Following Thouless and Guinness,[19] agonistic interactions were considered as occasions when one hind physically attacked another, or made a ritualised gesture associated with aggressive action that caused the other animal to move away. Threats included one or more of the following behaviours: butting the body with the forehead, biting (usually directed towards the back or ears), kicking with the forelegs and chasing, and varied in intensity, sometimes being merely an intention movement where no actual contact was made.[20] The dominance ranking for each individual was calculated as a linear hierarchy by winner-loser outcome of interactions on Matman V.1.1.4 matrix manipulation and analysis program (Noldus, Wageningen, The Netherlands) as explained by de Vries.[21] This method was chosen since it can be applied to a small sample size, as we had, with significant results. The statistical significance of the linearity (h') of the dominance hierarchy was determined by a sampling process using 10 000 randomisations.[22] The dominance hierarchy was reorganised by a two-step iterative procedure (1000 sequential trials) to order individuals by first minimising the number of inconsistencies, and thereafter the strength of the inconsistencies. The linear hierarchy was transformed according

TABLE 1: Pearson's correlations among dominance index, body mass and age and the haematologic profile in a herd of 24 Iberian red deer hinds (*Cervus elaphus hispanicus*) during lactation

	Dominance index	Body mass	Age
Body mass	0.571**		
Age	0.752**	0.682**	
Red blood cell count (RBC)	−0.585**	−0.727**	−0.621**
Haemoglobin (Hgb)	−0.637**	−0.577**	−0.586**
Haematocrit (Hct)	−0.615**	−0.584**	−0.533**
Mean corpuscular volume (MCV)	0.460*	0.714**	0.623**
RBC distribution width (RDW)	ns	ns	ns
Mean corpuscular haemoglobin (MCH)	0.453*	0.738**	0.595**
Mean cell haemoglobin concentration (MCHC)	ns	ns	ns
White blood cell count (WBC)	−0.545**	−0.456*	ns
Lymphocytes count	−0.472*	−0.662**	−0.649**
Not lymphocytes count	ns	ns	ns
Platelet count	ns	ns	ns
Mean platelet volume (MPV)	ns	ns	ns

ns, not significant.

to the formula 1−(rank/n) (n=24). Therefore, the dominance index varied in the range (0, 1) (0=submissive; 1=dominant).

Statistical analyses

For descriptive values, mean±SD is indicated throughout the text. Pearson's correlations were performed as preliminary analyses in order to understand the relationships among the explanatory variables (dominance index, body mass and age); among themselves and with the studied haematologic variables. Since the haematologic variables were correlated with some, or with all, three explanatory variables, general linear models were used to clarify which of the independent variables best explained the observed haematologic profile. Twelve models were built, one for each haematologic variable studied, with dominance index, body mass and age entered as covariates. The three variables were checked for multicollinearity due to them being correlated, but the maximum VIF obtained was 1.918 (see Table 1). All the variables were also normally distributed; the Shapiro-Wilk test was used, which fits better for smaller sample sizes. Non-significant variables were removed from the models in a backward stepwise procedure, until all the remaining variables were significant. Analyses were performed using IBM SPSS Statistics (V.20.0 for Windows, IBM, USA).

Results

The mean age of the animals studied was 6.1±3.3 years old (range: 2–12 years), and the mean body mass was 107.4±14.9 Kg (range: 75.1–136.9). All of the measured haematologic parameters were in the range reported for adult hinds of the species (Teare; not shown for concision purposes). Dominance index, body mass and age were positively correlated with MCV and MCH, and negatively with RBC, Hgb, Hct and lymphocyte counts. WBC counts were negatively correlated with dominance index and body mass, but not with age (Table 1).

General linear models further clarified these relationships (Table 2). Dominance index was the main variable influencing Hgb (R^2=34.4 per cent), Hct (R^2=34.6 per cent) and WBC (R^2=32.0 per cent). Body mass was the main variable influencing RBC (R^2=56.2 per cent) and MCH (R^2=45.9 per cent). Age was the main variable influencing MCV (R^2=46.8 per cent), lymphocytes (R^2=44.8 per cent) and platelets (R^2=20.8 per cent). Finally, count of not

TABLE 2: General linear models showing the influence of dominance index, body mass and age on the haematologic profile of Iberian red deer hinds (*Cervus elaphus hispanicus*) during lactation

Dependent variable	Independent variables *	R^2 (%)	β	t	P value
Red blood cell count (RBC)	*Intercept* Body mass	56.2	15.390±1.216 −0.058±0.011	12.651 −5.195	<0.001
Haemoglobin (Hgb)	*Intercept* Dominance index	34.4	16.513±0.348 −1.907±0.575	47.439 −3.315	0.003
Haematocrit (Hct)	*Intercept* Dominance index	34.6	43.301±0.891 −4.905±1.472	48.601 −3.332	0.003
Mean corpuscular volume (MCV)	*Intercept* Age	46.8	39.757±1.402 0.874±0.203	28.348 4.299	<0.001
RBC width (RDW)	–				
Mean corpuscular haemoglobin (MCH)	*Intercept* Body mass	45.9	9.512±1.832 0.071±0.017	5.192 4.218	<0.001
Mean cell haemoglobin concentration (MCHC)	–				
White blood cell count (WBC)	*Intercept* Dominance index	32.0	7.079±0.379 −1.967±0.626	18.699 −3.145	0.005
Lymphocytes count	*Intercept* Age	44.8	3.226±0.248 −0.149±0.036	12.999 −4.129	<0.001
Not lymphocytes count	*Intercept* Dominance index Age	29.9	3.763±0.364 0.177±0.070 −2.131±0.758	10.326 2.527 −2.810	0.011 0.020
Platelet count	–				
Mean platelet volume (MPV)	–				

*Dashes indicate that no significant model was obtained.

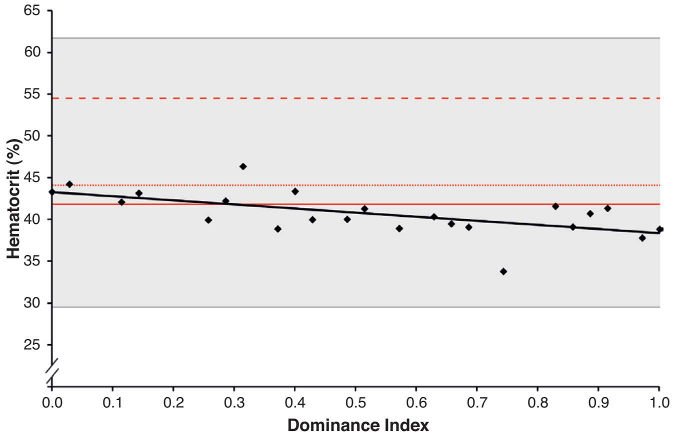

FIG 1: Correlation between dominance index and haematocrit (g/dL) concentration in a herd of Iberian red deer hinds (*Cervus elaphus hispanicus*). Reference values are: straight red line— mean for *C elaphus* hinds according to Teare; grey area— reference interval for *C elaphus* hinds according to Teare; broken red line—mean for physically restrained females of *C elaphus hispanicus* according to Marco and Lavín[2]; dotted red line—mean for chemically restrained *C elaphus hispanicus* according to Marco and Lavín.[2]

lymphocytes was attributed to dominance index and age (R^2=29.9 per cent). Since this study is focused on the effect of social rank (dominance index) on the haematologic profile, the correlations highlighted in the analyses are shown in Fig 1 (Hgb), Fig 2 (Hct) and Fig 3 (WBC), together with previously reported means and ranges.[2]

Discussion

While determining the haematologic profile is a useful tool for monitoring the health status of deer herds,[1 23 24] it is also necessary to take into consideration the factors that affect them. Our study confirmed several previously reported relationships, and importantly, our new hypothesis that social rank affects the haematologic profile. The influence of age has been well studied, and effects on RBC count, Hgb,[25 26] MCV[25–27] and lymphocyte count (negative effect reported by Upcott

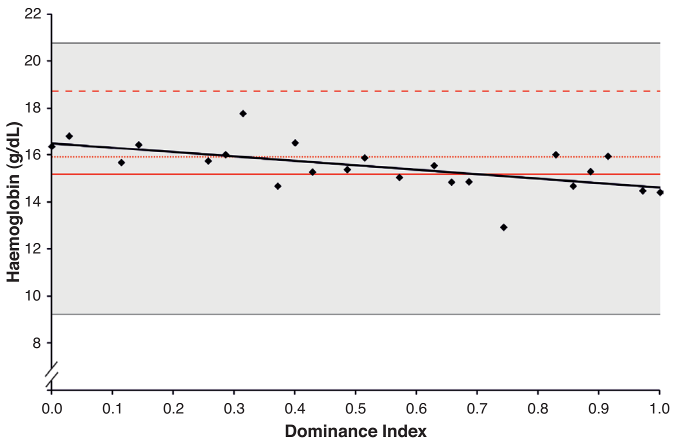

FIG 2: Correlation between dominance index and haemoglobin (per cent) concentration in a herd of Iberian red deer hinds (*Cervus elaphus hispanicus*). Reference values are: straight red line—mean for *C elaphus* hinds according to Teare; grey area—reference interval for *C elaphus* hinds according to Teare; broken red line—mean for physically restrained females of *C elaphus hispanicus* according to Marco and Lavín[2]; dotted red line—mean for chemically restrained *C elaphus hispanicus* according to Marco and Lavín.[2]

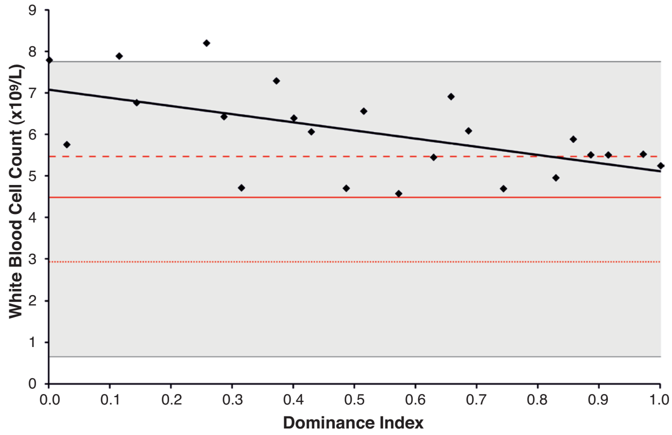

FIG 3: Correlation between dominance index and white blood cell count ($x10^9$/L) concentration in a herd of Iberian red deer hinds (*Cervus elaphus hispanicus*). Reference values are: straight red line—mean for *C elaphus* hinds according to Teare; grey area—reference interval for *C elaphus* hinds according to Teare; broken red line—mean for physically restrained females of *C elaphus hispanicus* according to Marco and Lavín[2]; dotted red line—mean for chemically restrained *C elaphus hispanicus* according to Marco and Lavín.[2]

and Herbert[28] and Mohri and others[29]; positive effect reported by McAllum[30]) are understood across cervid species. In general, our results agreed with the studies regarding the effect of age. It has however recently been pointed out that the relationship between age and WBC parameters in deer is not so clear.[1] Most cervids are social animals and generally there is a correlation between age and social rank (Table 1).[17] Our results clearly showed that the effect of social rank and WBC count is stronger than that of age, which suggests that previous results may have been misinterpreted due to the unrecognised effect of social rank. Also, few relationships between haematologic profile and body mass have been described (only with RBC[31]). Our data show that body mass is the main factor influencing the variability observed only in RBC count and MCH.

The main goal of this study was to highlight the relationship between haematologic profile and social rank. In the case of erythrocytes, we proposed two physiological mechanisms to explain how they may be affected by social rank. The first predicts greater RBC counts in dominant animals due to increased erythropoietic activity mediated by greater androgen levels.[7 8] This should happen even during lactation when hinds have low RBC counts.[24] However, this relationship was negative, and negative correlations also appeared with Hgb and Hct while MCV and MCH were positive. Nonetheless, the most important relationships according to models are those with Hgb (Fig 1) and Hct (Fig 2). Thus, this physiological mechanism was not supported, since the relationship was negative. The second proposed mechanism was lower RBC count in submissive animals due to increased stress and glucocorticoids level, which is supported by our results. In fact, our results are similar to those described when comparing chemically restrained deer (low stress) with physically restrained ones (high stress): RBC and WBC counts, Hgb and Hct are consistently higher in physically restrained animals.[1 2] It has been also observed that routine handling may reduce this 'stress-effect' by 10 per cent–20 per cent,[32] comparative to the differences observed among our more dominant and more submissive animals. We can therefore conclude that social stress in submissive animals may have a chronic effect as a long-term stressor on the haematologic profile. The effect of social rank on WBC count has not been reported previously in cervids but is well documented in pigs[33] and other vertebrates (reviewed in the study by Engler and others[34]).

As can be observed in the figures, our animals produced values within the reference and other published data.[35] WBC count values resembled those reported for physically restrained.[2] Our animals were subjected to routine weekly handling, which has been reported to lead to habituation to handling and low stress measured by behavioural indicators.[15] Overall, this means that our results and conclusions are not affected by other stress factors, and further confirms social stress as the main explanation for the data obtained.

Adaptive and ecological implications

In general, our results agree with those previously reported in other mammals, suggesting an underlying mechanism that has been conserved during evolution.[34] As they have been conserved, these effects of stress on complete blood count may be beneficial to the herd. Regarding Hgb and Hct, it could be argued that submissive hinds need to be more ready to escape at high speed, which requires a greater supply of oxygen to the cells; in comparison, dominant hinds rarely have to use reactions requiring bursts of oxygen supply. Regarding WBC, the effect found may be mediated (or reinforced) by nutrition: immune system needs are covered only after other needs such as maintenance or reproduction have been fulfilled.[10] That means that diet protein is used for milk production with higher priority than for the immune system, especially under food restriction situations.[11] Social rank determines the access to food in hinds, and allows them to select feed with a greater nutritional value.[36] Thus, subordinate hinds may have less resources for immune barriers, being forced to use internal immunity (WBC; similar results were found in restricted feed experiments in the study by Landete-Castillejos and others[37]). In this way, dominant hinds benefit from lower energy expenditure in WBC, while submissive animals benefit from enhanced defenses.

Conclusions and future steps

In summary, since WBC count, Hgb and Hct are clearly affected by social rank, we propose that social rank must be taken into consideration in the interpretation of the haematologic profile of red deer, and probably also for other social cervids and ungulates. Similar studies should be conducted in males, for which hierarchies are based on α-male structures much more intense during rut than outside the mating season. Increase of RBC count mediated by testosterone levels must be also tested in males during rut, even if this hypothesis was discarded in hinds.

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Competing interests None declared.

Ethics approval All experimental procedures were conducted under the approval of the Universidad de Castilla-La Mancha Ethics Committee.

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