Social rank affects the haematologic profile in red deer hinds

Francisco Ceacero,1 Enrique Gaspar-López,2,3 Tomás Landete-Castillejos,2,3,4 Laureano Gallego,3 Andrés J García2,3,4

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 Boes1 for an exhaustive summary), since it is almost impossible to obtain representative resting blood values. According to Wilson and Pauli3 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 others4 for pigs). Social rank may also affect the RBC count, as submissive animals are socially stressed5 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.
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 and it has been suggested that immune system requirements are covered only after other needs for maintenance, lactation or reproduction have been fulfilled. 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 m², 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 while animals were standing inside a small handling box (2m×2m×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. 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, 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, 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.

**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, 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. All interactions were recorded avoiding interferences in the behaviour of the animals, according to the focal group sampling method. The observer remained hidden from the animals outside the enclosure, with optimal observation conditions. Following Thouless and Guinness, 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. 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. 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. 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...
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 (R²=34.4 per cent), Hct (R²=34.6 per cent) and WBC (R²=32.0 per cent). Body mass was the main variable influencing RBC (R²=56.2 per cent) and MCH (R²=45.9 per cent). Age was the main variable influencing MCV (R²=46.8 per cent), lymphocytes (R²=44.8 per cent) and platelets (R²=20.8 per cent). Finally, count of not

---

**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

<table>
<thead>
<tr>
<th>Dominance index</th>
<th>Body mass</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>0.571**</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.752**</td>
<td>0.682**</td>
</tr>
<tr>
<td>Red blood count</td>
<td>−0.585**</td>
<td>−0.727** −0.621**</td>
</tr>
<tr>
<td>Haemaglobin</td>
<td>−0.637**</td>
<td>−0.577** −0.586**</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>−0.615**</td>
<td>−0.584** −0.533**</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>0.460*</td>
<td>0.714** 0.623**</td>
</tr>
<tr>
<td>RBC distribution width</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Mean cell haemoglobin concentration</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>White blood count</td>
<td>−0.545**</td>
<td>−0.456* ns</td>
</tr>
<tr>
<td>Lymphocytes count</td>
<td>−0.472*</td>
<td>−0.662** −0.649**</td>
</tr>
<tr>
<td>Platelet count</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Mean platelet volume</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, not significant.

**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

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>R² (%)</th>
<th>t</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell count (RBC)</td>
<td>Intercept, Body mass</td>
<td>56.2</td>
<td>12.651</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haemoglobin (Hgb)</td>
<td>Intercept, Dominance index</td>
<td>34.4</td>
<td>47.439</td>
<td>0.003</td>
</tr>
<tr>
<td>Haematocrit (Hct)</td>
<td>Intercept, Dominance index</td>
<td>34.6</td>
<td>48.601</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>Intercept, Age</td>
<td>46.8</td>
<td>28.348</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC distribution width (RDW)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin (MCH)</td>
<td>Intercept, Body mass</td>
<td>45.9</td>
<td>5.192</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean cell haemoglobin concentration (MCHC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell count (WBC)</td>
<td>Intercept, Dominance index</td>
<td>12.0</td>
<td>18.699</td>
<td>0.005</td>
</tr>
<tr>
<td>Lymphocytes count</td>
<td>Intercept, Age</td>
<td>44.8</td>
<td>12.999</td>
<td>0.001</td>
</tr>
<tr>
<td>Not lymphocytes count</td>
<td>Intercept, Dominance index, Age</td>
<td>29.9</td>
<td>10.126</td>
<td>0.011</td>
</tr>
<tr>
<td>Platelet count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dashes indicate that no significant model was obtained.*
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 MCV25–27 and lymphocyte count (negative effect reported by Upcott and Herbert28 and Mohri and others29; positive effect reported by McAllum30) 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).37 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 RBC31). 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,\textsuperscript{12} com-
parative to the differences observed among our more
dominant and more submissive animals. We can there-
fore conclude that social stress in submissive animals
may have a chronic effect as a long-term stressor on the
haematological profile. The effect of social rank on WBC
count has not been reported previously in cervids but
is well documented in pigs\textsuperscript{33} and other vertebrates (re-
viewed in the study by Engler and others\textsuperscript{34}).

As can be observed in the figures, our animals pro-
duced values within the reference and other published
data.\textsuperscript{35} WBC count values resembled those reported for
physically restrained.\textsuperscript{2} Our animals were subjected to
routine weekly handling, which has been reported to
lead to habituation to handling and low stress meas-
ured by behavioural indicators.\textsuperscript{15} Overall, this means
that our results and conclusions are not affected by oth-
er 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.\textsuperscript{34}
As they have been conserved, these effects of stress on
complete blood count may be beneficial to the herd. Regard-
ning Hgb and Hct, it could be argued that submis-
sive 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
by 5beta-H steroid metabolites.\textsuperscript{35} Thus, subordinate hinds may have a chronic effect as a long-term stressor on the
immune system, especially under food restriction
mechanism that has been conserved during evolution.\textsuperscript{34}

Increase of WBC count, Hgb and Hct are clearly
mediated by testosterone levels must be also
tested in males during rut, even if this hypothesis was
discarded in hinds.

Acknowledgements
The authors wish to thank Fulgencio Cebrián and Isidoro
Cambronerio (in memoriam) for help with animal handling, Jan Pluňáček for support with data from the International Species Information System, Chris Johnson for
professional language editing and the Complejo Hospitalario Universitario de Albacete
for processing the blood samples.

Funding
This study was supported by the grants IGA-20175014 (Faculty of Tropical
AgrSciences, Czech Republic) and RVC-2016-5327-2 (MINECO, Spain).

Competing interests
None declared.

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

© British Veterinary Association (unless otherwise stated in the text of the article)
2018. All rights reserved. No commercial use is permitted unless otherwise expressly
granted.

References
1. BOEKS KM. Hematologia de Cerdos. In: WEISS DJ, WARDROP KJ, eds. Veterinary hema-
2. MARCO I, LAVÍN S. Effect of the method of capture on the haematology and blood
3. WILSON PR, PAULJ IV. Blood constituents of farmed red deer (Cervus elaphus). II:
4. HJARVARD BM, OLE N. A., JUUL-MADSEN HR, et al. Social rank influences the distri-
bution of blood leukocyte subsets in female growing pigs. Scand J Lab Anim Sci
5. THULESS CR. Feeding competition between gazelle red deer hinds. Anim Behav
6. BOUSSIOL MF. Androgens, aggressive behaviour and social relationships in higher
7. GORDON AS, ZANANI ED, LEVERE RD, et al. Stimulation of mammalian erythropoiesis
8. GORSHENIN D, GARDNER PH. Erythropoietic activity of steroid metabolites in mice. Proc
11. HIOUDIK JG, KYRAZIYIAS I, JACSON F, et al. The relationship between protein nutri-
tion, reproductive effort and breakdown in immunity to Teladorsagia circumcincta in
12. INGRAM JR, CROCKFORD IN, MATTHEWS LR. Ultradian, circadian and seasonal rhythms
in cortisol secretion and adrenal responsiveness to ACTH and yarning in unrestrained
parameters and reticulocytes indexes on the Adiva A120 hematologic analyzer. J Lab
Veterinary haematology. Lippincott Williams and Wilkins:3–11.
15. CEACERO F, LANDETE-CASILLEJOS T, BARTOSOYA I, et al. Habituating to handling:
16. ASAB. Guidelines for the treatment of animals in behavioural research and teaching.
17. CEACERO F, LANDETE-CASILLEJOS T, GARCÍA AJ, et al. Kinship discrimination and
effects on social rank and aggressiveness levels in ibervan red deer hinds. Ethology
18. ALTMANN J. Observational study of behavior: sampling methods. Behaviour
19. THULESS CR, GUINNESS FE. Conflict between red deer hinds: the winner always wins.
Anim Behav 1986;34:1166–71.
20. HALL MJ. Social organization in an encircled group of red deer (cervus elaphus l.)
on rhum. i. the dominance hierarchy of females and their offspring. J Zoot psychol
21. DE VRIES H. Finding a dominance order most consistent with a linear hierarchy: a
22. DE VRIES H, VRIES, H DE. An improved test of lineality in dominance hierarchies
containing unknown or tied relationships. Anim Behav 1995;50:1375–89.
23. KLEIN KA, CLARK C, ALLEN AL. Hypoglycemia in sick and moribund farmed elk calves.
24. ZOIBORSZEY Z, HORN I, TIBOLY S, et al. Some haematological and immunological
Sydney, Australia: University of Sydney, 1989.
27. PEINADO V. I, CLEIRAN JR, PALMEQUE J. Basic haematological values in some
wild ruminants in captivity. Comp Biochem Physiol A Mol Integr Physiol

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 haematological 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.
Social rank affects the haematologic profile in red deer hinds

Francisco Ceacero, Enrique Gaspar-López, Tomás Landete-Castillejos, Laureano Gallego and Andrés J García

Veterinary Record published online January 26, 2018

Updated information and services can be found at:
http://veterinaryrecord.bmj.com/content/early/2018/01/25/vr.104629

These include:

References
This article cites 32 articles, 5 of which you can access for free at:
http://veterinaryrecord.bmj.com/content/early/2018/01/25/vr.104629#ref-list-1

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/