The immune challenge of mating effort: steroid hormone profile, dark ventral patch and parasite burden in relation to intrasexual competition in male Iberian red deer

Short running title: Immune challenge of mating effort in deer.

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Abstract

Testosterone secretion may regulate the reproductive effort and the development of sexual traits, but it may also involve costs at the immunological and metabolic levels. However, the evidence for this trade-off in wild populations is scarce. Similarly, cortisol also plays an important role in mediating the reproductive and immune functions. In this study, we analyzed whether the endoparasite burden relates to hormonal levels (fecal testosterone and cortisol metabolites) and/or morphological sexual traits (size of the dark ventral patch, a trait that indicates reproductive effort in males) in male Iberian red deer (*Cervus elaphus hispanicus*). For this purpose, we sampled male red deer harvested during hunting actions in two types of populations in southwestern Spain that differed in structure affecting the level of male-male competition for mates. We used coprological analyzes to estimate the parasite burden mainly of gastrointestinal and bronchopulmonary nematodes and of protozoa, and assessed testosterone and cortisol metabolite levels from fecal pellets. We found a positive relationship of host parasitisation with both testosterone levels and the size of the dark ventral patch, but these relationships depended on the intensity of male-male competition in the population, being only found under the high-competition level. These results are discussed under the hypothesis of the testosterone immunocompetence handicap, suggesting a cost at the immunological level, and, therefore, higher susceptibility to parasite infection in males that make a greater reproductive effort. However, this effect seems to be modulated by the social environment (male-male competition) that might lead to different optima in testosterone production and sexual trait development.

Key words: Dark ventral patch, intrasexual competition, fecal testosterone metabolites, male Iberian red deer, parasite burden.

Introduction

Steroid hormones are decisive in the regulation of male-male competition and reproductive behaviors in many polygynous species (Bronson & Desjardins, 1971; Wingfield *et al.*, 1987). Intrasexual competition usually implies aggressive behaviour, and testosterone is decisive in its regulation since its secretion is correlated with the defence of both territories and mates (Wingfield, 1997; Arteaga *et al.*, 2008), as well as with the expression of sex traits and reproductive effort. Moreover, male-male competition and its inherent agonistic interactions, often lead to the establishment of a hierarchy in the population, which is a source of allostatic load and stress (Creel, 2001; Creel, 2005). The higher the exposure to agonistic encounters, the greater the endocrine response to the stress by the hypothalamus-pituitary-adrenal axis, which promotes the secretion of glucocorticoid hormones (Stewart, 2003).

The handicap principle suggests that exaggerated secondary sexual traits can be an index of good health if they are costly to produce (Zahavi 1975). Hamilton & Zuk (1982) developed the immunocompetence handicap hypothesis, suggesting that the males' ability to face parasites and pathogens would affect their ability to invest resources in sexual signals and sexual traits (e.g. Roberts *et al.* 2003). The immune response is costly and usually conflicts with other physiological and behavioral requirements, which forces individuals to make trade-off. Especially, there is an important conflict between immune function and the males' reproductive behaviors (Sheldon & Verlhust 1996; Westneat & Birkhead 1998; Lochmiller & Deerenberg 2000). Hence, many studies have focused on the evolution of the relationship between parasites and the immune system physiology with reproductive behavior across species (Schmid-Hempel 2003; Viney *et al.* 2005). Reproductive effort, defined as the allocation of energy resources to current reproduction, is traded off against the

individual's ability to defend itself from pathogens (reviewed in Lochmiller & Deerenberg 2000; Zuk & Stoehr 2002; but see Hřrak *et al.* 2006; Corlatti *et al.* 2012). The physiological basis of this trade-off includes the role of the immune response. The steroid hormone testosterone may regulate male reproductive effort (Knapp 2003; Hirschenhauser & Oliveira 2006) and enhance mating potential (Folstad & Karter 1992; Ezenwa *et al.* 2012; but see Nie *et al.* 2012), while it simultaneously impairs the functioning of the immune system (e.g., Decristophoris *et al.* 2007).

Females should benefit from mating with males that have high testosterone levels, because only genetically high-quality males can afford or tolerate the costs of a decreased defence against parasites and pathogens induced by higher levels of testosterone (Zuk *et al.* 1995; Peters 2000). Thus, most of the predictions of the immunocompetence handicap hypothesis have focused on the traits involved in the choice of a mate. However, the immunocompetence handicap hypothesis may also apply to traits related to intrasexual competition (Roberts *et al.* 2004) and the reproductive effort.

Numerous studies have shown parasitism biased towards males. In a polygynous mating system, the male invests in secondary sexual characters and is involved in agonistic interactions, even decreasing food intake during the breeding season. Indeed, a positive correlation between reproductive effort and parasitism has been already revealed in various taxa (e.g. *Taeniopygia guttata*, Deerenberg *et al.* 1997; *Ficedula albicollis*, Nordling *et al.* 1998; *Tachycineta bicolor*, Ardia *et al.* 2003; *Passer domesticus*, Buchanan *et al.* 2003; *Lagopus lagopus scotica*, Mougeot *et al.* 2004; *Ovis canadensis*, Pelletier *et al.* 2005; *Homo sapiens*, Rantala *et al.* 2012). In ungulate species, there is a close link between parasite burden, testosterone levels and male dominance rank (*Rangifer tarandus tarandus*, Gaudernack *et al.* 1984; *Odocoileus*

virginianus, Ditchkoff *et al.* 2001; *Rupicapra rupicapra rupicapra*, Hoby *et al.* 2006, Corlatti *et al.* 2012; *Capra ibex*, Decristophoris *et al.* 2007).

The red deer (*Cervus elaphus*) is a highly polygynous species (Clutton-Brock *et al.* 1988), in which males strongly compete for females by defending either harems or mating territories (Carranza *et al.* 1990; 1995; 1996). The rutting season is an exhausting time. Males barely eat, they display a very high activity, chase each other and lose weight (Clutton-Brock *et al.* 1982; Carranza *et al.* 1995). The greater investment in rutting activities by territorial males suggests potentially high mating benefits (Carranza *et al.* 1996). Investment in sexual characters and reproductive effort, as well as the unpredictable agonistic interactions, should result in costs in terms of physiological stress (de la Peña *et al.* unpublished data). Red deer antlers constitute an important sex trait, widely studied, with high production costs (Goss, 1983). Antler size is positively correlated with winning fights and mating success (Clutton-Brock *et al.* 1982; Malo *et al.* 2009). In addition to the widely studied antlers, there is another non-permanent sexual trait in male Iberian red deer: the dark ventral patch (Martín *et al.* 2014; de la Peña *et al.*, 2019; Carranza *et al.* in preparation). The size of this trait reveals the dominance rank and reproductive effort that a male has made in the most recent rutting season (Martín *et al.* 2014; de la Peña *et al.*, 2019). This information is important both for females during the mate choice processes, and for other males in intrasexual competition.

Antler length, age and testosterone metabolite levels are associated with the size of the dark ventral patch (de la Peña *et al.* unpublished data). As in other species of ungulates, such as roe deer (Corlatti *et al.* 2012), the greater investment in rutting activities suggests potentially high mating benefits. However, the secretion of androgen

hormones and the high reproductive effort during the mating season could compromise health state and, therefore, lead to a decrease in parasite resistance.

Previous studies in Iberian red deer show a greater prevalence or intensity of parasite burden in males with respect to females (Ruiz-Fons *et al.* 2013), suggesting a cost derived from investment in the immune system during the mating season. Malo and colleagues (2009) showed a positive relationship between testosterone metabolite levels and endoparasite burden in male Iberian red deer. However, there is a lack of studies showing whether the actual reproductive effort can play a fundamental role in the health state of males and the susceptibility to parasite infection. The greatest investment during the mating season involves numerous behaviors that compromise the individual's health and fitness. A higher rate of roaring and a greater number of agonistic interactions, as well as a reduction of food intake, imply the maintenance of high levels of steroid hormones, such as testosterone and cortisol. However, the extent to which rutting males are involved in actual competition with rival males depends on the structure of the population, including sex ratio and age structure of males. In southern Spain, there are two types of red deer populations differing in age-sex structure and in the level of male-male competition for mates (see below, Methods section). This fact allows the study of the role of mate competition in the relationship between steroid hormones, sex traits and parasite burden.

Thus, the aim of this study was to investigate the relationship between endoparasite burden and testosterone hormone levels as well as the effect of investing in a bigger ventral patch as a reproductive effort signal in free-ranging male Iberian red deer. Our hypothesis is that males with a large dark ventral patch and elevated testosterone metabolite levels will "suffer" a cost to their immune system, thus making

them more susceptible to parasite infection, but that this relationship may be modulated by the actual level of male-male competition in the population.

Methods

Study area and red deer populations

We collected data from 217 male Iberian red deer harvested during hunting activities between October and February, during two consecutive hunting seasons (2015-2017). Hunting took place in 14 areas (hunting states) located in southwestern Spain, in the regions of Andalusia (Sierra Morena, Córdoba province) and Extremadura (Sierra San Pedro, Badajoz and Cáceres provinces), within the natural distribution range of this species. Each area, with an average surface of 2347 ha, constitutes a population due to the existing of natural or artificial barriers, or because of the geographical distance between them (a mean of around 25 km). Red deer density is around 0.3 individual/ha (ranges = 0.1-1.0 indiv. /ha) (J. Carranza, University of Extremadura, unpublished data). The study area comprises a mountain range covered by open oak woodland known as 'dehesa' in addition to Mediterranean shrubs such as *Cistus* spp., *Erica* spp., *Arbutus unedo*, *Phyllirea* spp., *Genista hirsute* or *Lavandula* spp.

There are two different types of management in these hunting estates depending on whether the state is fenced or not with a hunting mesh, which does not allow the free movement of deer between estates (Pérez-González & Carranza 2009; Torres-Porras *et al.* 2014). These differences in management and in hunting pressure have consequences on the sex and age structure of the deer population, but not on the population densities (Torres-Porras *et al.* 2014). In unfenced estates, there is a bias towards mature females and males compared to fenced estates (Pérez-González & Carranza 2009; Torres-Porras *et al.* 2014). This may be considered as an "experiment under natural conditions" where

there are two types of populations with different pressure and intensity of male-male competition for mating (Pérez-González & Carranza 2009, 2011; Pérez-González *et al.* 2012). In fenced states, males experience a high level of intra-sexual competition compared to that of unfenced estates, where all males can mate even if they are sub-adult (Pérez-González & Carranza 2009, 2011; Pérez-González *et al.* 2012). For this reason, we have established two categories in relation to the level of male-male competition: high competition (HC; N= 66) population refers to fenced estates, while low competition (LC; N= 151) population refers to unfenced estates.

Data collection

We took morphological measurements *in situ* from deer within a few hours after being shot. We measured the maximal length of the dark ventral patch of male deer (Fig. 1). Antler size was calculated from the mean length of the left and right antlers, measured from the burr to the most distal tine. The jaws of each individual were collected to estimate age by counting cementum growth marks at the interradicular pad under the first molar as described by Carranza *et al.* (2004).

We conducted hormone levels quantification by detecting testosterone and cortisol metabolites in fecal samples collected from the rectum of each individual and frozen at -20°C until laboratory analyses. This is a widely used and validated procedure (Barja *et al.* 2007; Barja *et al.* 2008; Escribano-Ávila *et al.* 2013; Piñeiro *et al.* 2012; Iglesias-Merchán *et al.* 2018), useful to obtain information on the existing hormone circulating levels 12 to 24 hours before sampling (Barja *et al.* 2012). In the laboratory, frozen faecal samples were dried and pulverized. We took 0.5 g of faeces and added 2 ml of phosphate buffer and 2 ml of 100% methanol. The mixture was vortexed. After that, samples were in the shaker for 16 h. The solvent was decanted and the supernatant was centrifuged at 4000 rpm during 30 min. We determined the faecal metabolite

hormone levels using commercial enzyme immunoassay kits. The intra-assay coefficient of variation of the testosterone was 10.8% and inter-assay 10.6%. The intra-assay coefficient of variation of cortisol was 9.2% and inter-assay 10.2%. Standard dose-response curves were constructed by plotting the binding percentage ($B/B_0 \times 100$) against the standard hormone concentrations were added. Fecal hormone metabolite levels (FTM: fecal testosterone metabolite levels; FCM: fecal cortisol metabolite levels) are presented as nanogram per gram of dry fecal matter.

Parasitological analyses

Parasitological analyses were performed in the Parasitology Department of the Veterinary Faculty of the University of Córdoba (Córdoba, Spain). Following MAFF (1986), fecal egg, larvae and oocyst count were made in duplicate.

Fecal samples were analysed by a zinc sulphate-based flotation method using the McMaster chamber. Briefly, 3 g of faeces were thoroughly mixed with 42 ml of saturated $ZnSO_4$ solution (specific gravity = 1.35) and parasitic dissemination elements (eggs, larvae and oocysts) were counted using a modified McMaster method from the MAFF (1986) protocol. The flotation techniques in saturated $ZnSO_4$ were used to detect eggs of gastrointestinal nematodes, trematodes and cestodes, coccidian oocysts as well as bronchopulmonary nematode larvae in each fecal sample (see Cringoli *et al.* 2010). Prevalence is presented in percentage of the number of fecal samples with one or more specimens of a parasite genus in relation to the total number of fecal samples examined (Bush *et al.* 1997). Parasite burden was estimated as the number of a parasitic form (oocysts, eggs, or larvae) in a single fecal sample (Bush *et al.* 1997). Results were expressed as mean of eggs, oocysts of larvae per gram of faeces.

The susceptibility of the host to parasitic infections and its ability to respond to parasite infections could impact the egg count (Beldomenico & Begon 2010). However,

based on the high correlation reported in roe deer between fecal egg count and the number of adult parasites in the digestive tract (Appendix 1 in Body *et al.* 2011), fecal egg count can be assumed to be reliable markers for parasite infections.

Previous studies of deer parasites (Vicente *et al.* 2005) have found that, early in autumn, there is a peak of the bronchopulmonary nematode larvae *Elaphostrongylus cervi*, with the excretion output coinciding with early rains. Even though Vicente *et al.* suggest that the *E. cervi* L1 (*EcL1*, first-stage larvae) infection rates of the animals sampled during the hunting season in Mediterranean Spain may be comparable if the results are adjusted according to seasonal variation. For this reason, we collected from the Climate Research Unit (CRU) monthly rainfall data for the hunting season (October–February) for the areas where the samples were collected. (These data are available at <http://www.cru.uea.ac.uk/data>). The cumulative precipitation daily averages was calculated for each month of the two hunting seasons and for each population. The overall mean cumulative precipitation in the study areas was 1489 mm (range = 347-3436 mm).

Statistical analyses

We tested the relationship between the collection month and the frequency of parasitized and non-parasitized male deer using Pearson's Chi-square test with Yates' continuity correction with the package 'Stats'. Furthermore, we ran an one-way ANOVA analysis to measure the relationship between the frequency of parasitized and non-parasitized male deer and the cumulative rainfall per month in further analyses. Since we found correlations between both collection month and cumulative monthly rainfall, on one hand, and the number of parasitized males, on the other hand, we decided to include them as covariables.

We first used a Generalized Linear Model fitted to a binomial distribution without random effects (Table 3) to check the effect of the explanatory variables on the response variable “host parasitisation” (defined as presence/absence of all the parasitic forms found). Based on our starting hypothesis that the effort during the rut can decrease the effectiveness of the immune system and increase the parasite burden, and that, in turn, this can vary depending on the level of competition between males, we decided to include in the model the interactions between intensity of male-male competition and the variables related to this competition (the dark ventral patch and antler length, FTM and FCM).

We performed separate analyses of each parasite group (see Table 1). Because of the low prevalence of *Dictyocaulus* spp., we grouped it with *Elaphostrongylus* spp. even though it is an extrapulmonary lungworm larvae. We did not consider carrying out separate analyses of *Eimeria* spp. because of its low sample size. No trematode or cestode eggs were finally detected, so they were not analyzed. Thus, in the second and third models (Table 4 and 5), the dependent variables were the number of gastrointestinal fecal eggs and the bronchopulmonary fecal larvae count, respectively. We analyzed the interactions between the month when the samples were collected and the variables involved in the reproductive effort (such as the dark ventral patch size, antlers length, FTM and FCM) using an Ordinal Multinomial Mixed Model.

We added “population” and “region” as random factors to control for repeated sampling in the same population in different years. To facilitate model convergence, all quantitative variables were z-transformed, being the mean of zero and a standard deviation of one (using the *scale* function). We also considered the two-way interactions and, thus, to avoid risks of over-parameterization, we did not take into account non-

significant interactions from the full model. The sample size was 217 observations from 14 estates, some of them repeated along 2 years.

We used Generalized Linear Model and Ordinal Multinomial Mixed Model fitted by restricted maximum likelihood (REML) in R Studio software using the lme4 package (Bates *et al.* 2015). We also checked the homogeneity and independence of residuals of both models. In order to choose the model that explains the majority of the changes, an Information-Theoretic approach was used (Anderson & Burnham, 2002). To choose the most plausible models, we fitted the combination of all the variables using Akaike's information criteria (Anderson & Burnham 2002). First, we selected the best structure for the random effects (Table 2) by REML. We selected models with smaller AIC values, and we compared them using a likelihood ratio test. The means were given $\pm$ SE and the level of statistical significance was $P < 0.05$.

Results

Host parasitisation

The coprological analyses indicated the presence of endoparasites in 143 of the 217 (65.90 %) fecal samples analyzed. The McMaster protocol revealed the presence of eggs, oocysts, and larvae belonging to five different taxons of parasites (Table 1). Overall, gastrointestinal nematodes showed the highest prevalence (55.53%), followed by bronchopulmonary nematodes (27.18%) and protozoa (*Eimeria spp.*; 4.61%). None of the samples contained trematodes or cestodes eggs.

We found a correlation between the collection month and the number of parasitized males ($\chi^2 = 30.728$, $df = 4$, $p\text{-value} < 0.001$) and a marginal significance between cumulative monthly rainfall and the number of parasitized males ($F_{1,215} = 3.101$, $p\text{-value} = 0.080$).

The results of the first model (Table 3) showed a positive relationship between the fecal testosterone metabolite levels (FTM) and the dark ventral patch size on the presence of parasites (Table 3). The fecal cortisol metabolite levels (FCM), antler length, mate competition level and age had no significant relationship with the presence or absence of parasites in the host. In addition, two interactions were significant; first, the relationship between FTM and parasite burden was different between the two groups (LC and HC males) (Fig. 2A). The same happened between the dark ventral patch size and the presence of parasites (Fig. 2B). In both cases, under conditions of intense male-male competition (HC), there was a positive relationship between the presence of parasites and both FTM and the dark ventral patch size. In contrast, in populations with low levels of male-male competition (LC), these relationships did not appear.

Gastrointestinal nematodes

The results derived from an Ordinal Multinomial Mixed Model showed that neither FTM, FCM, age or intensity of male-male competition were related to the gastrointestinal nematode burden (eggs per faeces gram; epg) (Table 4). However, the size of the dark ventral patch and antler length were positively and significantly related to gastrointestinal epg (GIN).

Three interactions with the collection month were significant: the interactions with antler length, dark ventral patch size, and cumulative monthly rainfall. We found a similar pattern in the interactions of these three variables; thus, the positive relationships between these variables and the gastrointestinal epg were stronger in November than in the other months during the hunting season (October–February) (Table 4; Fig. 3).

Bronchopulmonary nematode larvae

The number of bronchopulmonary nematode larvae per gram of faeces (lpg) did not vary randomly; concretely, the Ordered Logit Mixed Model indicated that age was the variable explaining the increase in lpg values (Table 5). Antler length showed a marginal relationship with the bronchopulmonary burden. More specifically, lpg values were significantly higher when individuals had smaller antlers. However, we found a significant positive relationship between the bronchopulmonary parasite burden and the age of male deer.

Discussion

The results support our hypothesis that increased testosterone levels and the development of a large dark ventral patch increases the male Iberian red deer's susceptibility to parasite infection. Our predictions were based on two factors: (a) the testosterone immunocompetence handicap, suggesting a cost at the immunological level, and (b) the greater susceptibility to parasite infection derived from the higher inversion in testosterone levels, and the greater reproductive effort of a male, signalled by the dark ventral patch size. The main result of this research is the positive relationship of both fecal testosterone metabolite levels and the dark ventral patch size with the host parasitisation. However, it is interesting to note that this relationship differs between populations with different intensity of male-male competition, which suggests that the social environment modulates the relationship between reproductive effort and parasite load.

The size of the dark ventral patch is positively related to testosterone metabolite levels (de la Peña *et al.* unpublished data): the greater energy investment during the rut and reproductive effort, the greater the fecal testosterone metabolite levels. This relationship is more intense when males are under a high competition situation than in a low one (de la Peña *et al.* unpublished data). This suggests that both variables are highly

influenced by the effort that stags have to make to increase their mating chances. The testosterone's dual effect has been widely tested in many different species. On the one hand, this reproductive hormone is involved in the development of sexual signals. On the other hand, testosterone may cause immunosuppression (i.e., the immunocompetence handicap hypothesis, Folstad & Karter 1992). In ungulate species, testosterone also plays an important role in reproduction, intrasexual competition behaviors (Brockman, *et al.* 2001; Rajagopal 2009), as well as in the development of secondary sexual characters (Malo *et al.* 2009). However, the evidence of the testosterone immunosuppressant effects in ungulates in free-ranging conditions is scarce (*Capra ibex*: Decristophoris *et al.* 2012; *Nanger granti*: Ezenwa *et al.* 2012; *Equus quagga*: Cizauskas *et al.* 2015; *Capra pyrenaica*: López-Olvera *et al.* 2015; *Rupicapra pyrenaica pyrenaica*: Martínez-Guijosa *et al.* 2015). Our results support the idea that the immunosuppressant effect of testosterone may explain the greater susceptibility of males to parasite infection. Moreover, males that made a great reproductive effort and suffered a higher intrasexual competition for mates had a greater susceptibility to parasite infection.

In this study, we did not find any effect of cortisol on the presence of parasites and parasitic burden despite the relevance of stress hormones on different functions. On the one hand, Gaspar-López *et al.* (2010) showed a decrease of cortisol levels during the rut in red deer, associated to the inhibitory effect of testosterone on cortisol release. On the other hand, chronic stress might lead to a drop in androgen levels (Cumming *et al.* 1983; Mooring *et al.* 2006; Mehta *et al.* 2008; Bartos *et al.* 2010). However, previous studies (de la Peña *et al.* unpublished data) showed that under a high male-male competition situation, the positive link between testosterone metabolite levels and stress hormone levels is more intense, due to the associated behaviors and stressful situations

related to the intrasexual competition. Thus, our results suggest that testosterone reduce cortisol release, which explains the lack of significance of FCM.

When we looked at different temporal windows within the sampling period, the results from the quantitative parasitological analyses showed that the size of the dark ventral patch and the antlers were positively related to the number of gastrointestinal nematode fecal eggs, but only in red deer males collected in November. Also, we found that the cumulative monthly rainfall had a positive relationship with the males' amount of parasite eggs only in November. Previous studies with other species also showed the effect of rainfall in gastrointestinal parasite infections (Ezenwa 2004; Turner *et al.* 2012). In November, coinciding with the early autumn rains, there was a gastrointestinal nematode peak, probably due to the fertility changes in adults. For this reason, males might have been more exposed to parasite infection during this period than in the rest of the sampling period. However, only those males with bigger dark ventral patches and larger antlers were highly parasitized by gastrointestinal nematodes. The reason may be that in November, immediately after the rutting season, dominant males might be more vulnerable to parasites after high reproductive effort. This agrees with the effect not only derived from their testosterone levels, but also from the male-male competition associated behaviors.

Our study supports the suppression of immunological defences derived from the reproductive effort, testosterone metabolite levels, and investment in sexual signals. These results are consistent with those found in male chamois (*Rupicapra rupicapra*; Corlatti *et al.* 2012), where the individuals that increased their mating effort and androgen levels also showed higher parasite levels during the rut. Fitness gain by red deer males due to their investment in the current mating season has been shown to be counter balanced by longevity costs derived from tooth wear (Carranza *et al.* 2004;

Perez-Barbería *et al.* 2015). Here we demonstrate that, in addition to the testosterone levels and sex traits, the social environment, regarding the mate competition level, also plays a role in the balance between present and future reproduction effort because they influence the animal's immune capacity, and a depressed immune response leads to parasite infection and disease, leading to longevity costs (Nussey *et al.* 2012, 2013).

In summary, our analysis has evidenced a trade-off between mating effort and parasite infection in male Iberian red deer, and that the proximate mechanism underlying this pattern may be found in the secretion of testosterone and the investment in the development of sexual traits such as a dark ventral patch, but also in the level of male-male competition endured by rutting males in their populations. Future studies should be aimed at demonstrating whether the costs at the immunological level suffered by the males that invest more during the reproduction (due either to intense male-male competition or to their dominance rank) reduce their physical body condition, increase disease development, and, as a consequence, compromise their longevity.

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Table 1. Endoparasites found (mean number/g faeces $\pm$ SE) in fecal samples of male Iberian red deer (N = 217) from south-western Spain.

Parasitic group	Genera		
		Larvae/g	Prevalence (%)
Bronchopulmonary nematodes	<i>Elaphostrongylus</i> spp.*	14.68 $\pm$ 2.41	24.88
	<i>Dictyocaulus</i> spp.	1.14 $\pm$ 0.50	2.30
		Eggs/g	Prevalence (%)
Gastrointestinal nematodes	Strongylids spp.	35.31 $\pm$ 3.12	49.31
	<i>Trichuris</i> spp.	2.99 $\pm$ 1.31	3.22
Trematodes	-	0	0
Cestodes	-	0	0
		Oocysts/g	Prevalence (%)
Protozoa	<i>Eimeria</i> spp.	6.90 $\pm$ 3.42	4.61

**Elaphostrongylus* spp. extrapulmonary lungworm.

Table 2. Results of the model selection procedure for the random effects structure.

Models are ranked from the most simple structure (including only fixed effects) to the most complex.

Random effects	df	AIC
No random effects	16	249.99
(1 Population)	28	267.85
(1 Region/Population)	29	269.85
(1 Region) + (1 Population)	29	269.85

Table 3. Generalized linear model testing the effect of FTM, FCM, antler length, dark ventral patch size, age and male-male competition situation on the host parasitiation (presence/absence). Significant results are indicated in bold.

Variables	F	df	<i>p</i> -value
Month	7.812	4	< 0.001
Rainfall	0.832	1	0.323
Testosterone (FTM)	4.555	1	0.034
Cortisol (FCM)	2.709	1	0.101
Antler length	0.880	1	0.349
Dark ventral patch size	4.420	1	0.037
Age	0.293	1	0.589
Mate competition	2.123	1	0.150
Testosterone × Mate competition	8.866	1	0.003
Dark ventral patch size × Mate competition	5.072	1	0.025

Table 4. Ordered Logit Mixed Model testing the effect of FTM, FCM, antler length, dark ventral patch size, age and male-male competition situation on the gastrointestinal parasite burden (eggs/g) fitted with Laplace approximation.

Variables	Estimate	SE	Z	df	p-value
Month	-0.629	0.211	-2.982	4	0.003
Testosterone (FTM)	-0.064	0.151	-0.426	1	0.670
Cortisol (FCM)	0.145	0.153	0.945	1	0.345
Antler length	1.432	0.617	2.322	1	0.020
Dark ventral patch size	-0.949	0.474	-2.001	1	0.045
Age	0.106	0.200	0.53	1	0.595
Mate competition	-0.696	0.593	-2.982	1	0.241
Rainfall	-1.874	0.782	-2.397	1	0.017
Month × Antler length	-0.444	0.183	-2.429	1	0.015
Month × Dark ventral patch size	0.408	0.169	2.418	1	0.015
Month × Rainfall	0.902	0.324	2.783	1	0.005

Random factor: *Population*: variance $\pm$ SE = 0.261 $\pm$ 0.511.

Table 5. Ordered Logit Mixed Model testing the effect of FTM, FCM, antler length, dark ventral patch size, age and male-male competition situation on the bronchopulmonary parasite burden (larves/g) fitted with Laplace approximation.

Variables	Estimate	SE	Z	df	p-value
Month	-0.758	0.378	-2.004	4	0.045
Testosterone (FTM)	-0.473	0.374	-1.265	1	0.206
Cortisol (FCM)	0.205	0.204	1.005	1	0.315
Antler length	-0.676	0.373	-1.903	1	0.057
Dark ventral patch size	0.149	0.241	0.617	1	0.537
Age	0.698	0.256	2.728	1	0.006
Mate competition	0.665	0.722	0.921	1	0.357
Rainfall	-0.782	0.442	-1.772	1	0.076

Random factor: *Population*: variance $\pm$ SE = 0.377 ± 0.614 .

Figure legends:

Figure 1: Photographs showing the section of two ventral patches of male Iberian red deer. The measurement of the length of each patch from the penis to the anterior limit of the dark surface is exemplified with a marked red arrow in both cases.

Figure 2: Host parasitization (parasitized or non-parasitized) in relation to (a) levels (ng/g of dry fecal matter) of fecal testosterone metabolites (FTM) and (b) the dark ventral patch size (cm) under low (LC) or high (HC) male-male competition.

Figure 3: Relationship between (a) the antler length (cm) or (b) the dark ventral patch size (cm) and the gastrointestinal fecal egg counts (GIN) along the collection month. GIN fecal egg counts refers to predicted values from the model (mean $\pm$ SE; Table 4).

Figure 1

Figure 2

(a)

(b)

Figure 3

(a)

(b)