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Individual stress responses to *Sarcoptes scabiei* infestation in Iberian ibex, *Capra pyrenaica*

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ABSTRACT

In this study we have monitored the stress of Iberian ibex at individual level within the course of an experimental infection with *Sarcoptes scabiei* mites. For this purpose we have measured faecal 11-ketoetiocholanolone (11-k) using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). We used linear mixed models to explore the effects of host sex and age, clinic (mange status) and time (number of days post-infection) on the concentration of faecal 11-k. The most parsimonious model included clinic, time and host age, which explained 76.6% of the variance of the response variable. Moreover, the concentration of faecal 11-k varied greatly between individuals. Our results evidence the stressor nature of the disease and highlight the negative effects on hosts due to cortisol release and activity.

1. Introduction

The impact of stressors or aversive stimuli on animals's health depends on their ability to cope with such a situation. This ability may differ between species and individuals (Koolhaas et al., 1999; Dobson and Smith, 2000; Cockrem, 2013). These differential individual responses are shaped by a wide variety of factors, such as genotype, sex, body condition, season, breeding status, development, early experience (e.g., maternal care), and social behaviour and support, among others (Meaney, 2001; Romero, 2002; Cockrem, 2013; Cox et al., 2016). Stress response is an adaptive and complex mechanism which promotes immediate survival through mobilizing energy stores triggered by the release of glucocorticoid hormones (GCs) mediated by the activation of the hypothalamic–pituitary–adrenal axis (HPA) (Reeder and Kramer, 2005). Since this response may be inherited (Meaney, 2001; Cox et al., 2016) it can also respond to selection pressures (Kim et al., 2018). GCs act on practically all organs and tissues and regulate virtually every component of the immune and inflammatory responses (Munck and Guyre, 1991; Griffin and Thomson, 1998). One of the most potent anti-inflammatory effects of GCs is the suppression of the mediators of the immune response (Munck and Guyre, 1991).

GC secretion is increasingly being used as an endocrine index of stress, cortisol being the main stress biomarker in ruminants (Touma and Palme, 2005). But some studies focussing on both domestic (Palme and Möstl, 1997) and wild species, such as roe deer, *Capreolus capreolus* (Dehnhard et al., 2001) evidenced that most of the cortisol and corticosterone are metabolized up to the point to becoming practically absent in faeces. This is mainly due to the microbial activity and could alter faecal GC measurement, particularly when working with non-fresh samples (Washburn and Millspaugh, 2002). So, non-invasive characterization of stress is focused on GC metabolites, such as 11-ketoetiocholanolone (11-k) which is less subject to degradation (Keay et al., 2006; Azorit et al., 2012).

Most studies on animal stress responses lack repeated samples from individual animals and/or mainly report and discuss mean responses for groups of animals (Cockrem, 2013; George et al., 2014). On the other hand, if sample collection (e.g. of blood, saliva or urine) involves the capture and handling of animals, measured GC concentrations may

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be higher than the basal (reference or "true") values (Reeder and Kramer, 2005).

Sarcoptic mange is a disease of mammals' skin, caused by the mite *Sarcoptes scabiei* worldwide. In Europe this disease affects several wild Bovidae species (Bornstein et al., 2001; Pence and Ueckermann, 2002), including the Iberian ibex (*Capra pyrenaica*). Mangy ibexes show a marked reduction in body weight, become anaemic and suffer secondary infections (Carvalho et al., 2015; Pérez et al., 2015). Moreover, internal organs in infested hosts also become affected by the disease (Espinosa et al., 2017b). In addition to these pathological changes, sarcoptic mange also negatively affects ibex reproduction (Sarasa et al., 2011; Espinosa et al., 2017a).

The epizootiology of sarcoptic mange (e.g. prevalence and related mortality) differs across geographic areas, host species and populations (Bornstein et al., 2001; Pence and Ueckermann, 2002). At the individual level, some hosts develop an inflammatory immune response with a protective effect against reinfection (Arlian et al., 1994; Arlian et al., 1996). Alasaad et al. (2013) reported only 20% of mortality among naturally-infested Iberian ibex from the Sierra Nevada mountain range (southern Spain), while the remaining animals (80%, n = 25) recovered completely and spontaneously from mange (that is, without any anthropogenic treatment).

Despite the seemingly stressor nature of sarcoptic mange, the magnitude of the host response through the release of glucocorticoid hormones (with immunosuppressive effect) is poorly known. In this study, taking advantage of an experimental infestation of Iberian ibexes to address the pathology caused by mites (Espinosa et al., 2017b), we monitored faecal 11-ketoetiocholanolone (11-k, one of the main cortisol metabolites) repeatedly in infected individuals. The aim of this study is to examine the effect of host sex and age, time after experimental infestation, and severity of *S. scabiei* infection on the stress host response.

2. Material and methods

2.1. Ibex capture and adaptation to captivity

In this study we used 39 Iberian ibexes (17 females and 22 males, ranging from one to eleven years of age) from the Sierra Nevada Natural Space (SNNS) (36°55′–37°10′ N, 2°56′–3°38′ W) and the Sierras de Cazorla, Segura y Las Villas Natural Park (SCSVNP) (37°53′-37°88′ N, 2°53′-2°88′ W). Age of animals was estimated by counting horn segments (Fandos, 1991). The animals were captured with a mixture of xylazine (3 mg/kg) and ketamine (3 mg/kg) (Casas-Díaz et al., 2011) and transferred to an experimental enclosure located in the Sierras de Húetor Natural Park (SHNP) (37° 18′-37° 30′N, 3° 28′-3° 47′W). The ibexes were distributed in groups (4–6 individuals), in separate enclosures, where they were able to move freely and had *ad libitum* access to water and food. The acclimatization period lasted 8 weeks. During the experiment they did not receive any anti-parasite treatment.

2.2. Experimental infestation

Twenty-five Iberian ibexes (11 females and 14 males) were experimentally infested with *Sarcoptes scabiei* for 131 days, whereas the other 14 ibexes (6 females and 8 males) were maintained as controls for the same period. Infestation was performed by attaching a skin fragment from a naturally-parasitized (scabietic) ibex, containing an estimated burden of 750 ± 440 mites, in the inter-scapular region (previously shaved) (Espinosa et al., 2017b). During the course of the experiment ibex were visually assigned to one of the following categories defined according to the percentage of affected skin surface: healthy (mange-free ibex), mild (initial and development stages, with lesions on < 50% of the host skin surface), and severe (consolidation and chronic stages with lesions affecting more than 50% of the host skin surface) (Pérez et al., 2011).

2.3. Samples collection and analysis

Fresh faecal samples were collected by manual removal from the rectum or immediately after deposition, labelled, stored in carbonic ice, and transported to the laboratory, where they were kept at −80 °C until metabolite extraction and analysis. Samples for this study were collected at 103 and 131 days post-infection. One of the main cortisol metabolites, 11-ketoetiocholanolone (11-k), was quantified from these faecal samples following the analytical method described by Molina-García et al. (2018). Briefly, it consisted of the extraction of faecal glucocorticoid metabolites (FGCM), precleaning, purification and preconcentration of FGCM extracts, and then analysis with high-performance liquid chromatography coupled with tandem mass spectrometry HPLC-MS/MS, which allowed an unequivocal determination of the 11-k concentration.

2.4. Statistical analyses

We used linear mixed models (LMM) for longitudinal data analysis (McCullagh and Nelder, 1989; Zuur et al., 2009) to explore the effects of host sex and age, clinic (mange status) and time (number of days post-infection) on the concentration of faecal 11-ketoetiocholanolone (11-k) at an individual level. Therefore, faecal 11-k concentration could be considered both as a response and a consequence of the disease, and as predictor of mange severity as well. In order to perform an appropriate fitting of the LMMs, we transformed the variables in the following way: faecal 11-k was the response variable and was log-transformed, and host sex and age, clinic (mange status) and time (number of days post-infection) were the explanatory variables. With regards to the mange status, the individuals were categorized as 0: healthy (control), 1: mild (initial and development stages with lesions on < 50% of host skin surface), and 2: severe (consolidation and chronic stages with lesions on more than 50% of host skin surface). In the models, the identity of each animal was included as a random factor in order to consider the inter-individual effects on the faecal 11-k.

We used R software 3.3.2 (R Development Core Team, 2017) to conduct all the statistical analyses. Package "lme4" (Bates et al., 2015) was used to fit LMMs, with the *lmer* function. Before model construction we evaluated whether the mange status was significantly influenced by post-infection by means of Friedman's test (Wayne, 1990) with the *friedman.test* function in R package "stats". We used a model-averaging approach that increases the robustness of the parameter estimates, accounts for model uncertainty, and provides a measure of the relative importance of each predictor variable (Burnham and Anderson, 2002; Grueber et al., 2011). Accordingly, we used the *dredge* and *model.avg* functions in R package "MuMIn" (Barton, 2016) to perform the model averaging analysis. The *dredge* function was used to construct a set of candidate models with all the possible combinations of predictive variables, and according to these, we identified the best models using an automatic selection procedure based on the Akaike Information Criterion corrected for small sample sizes (AICc) (Burnham and Anderson, 2002) provided by the *dredge* function. The most parsimonious model is that with the lowest AICc value. The models with a difference between their AICc values and the lowest AICc value greater than 2 units (ΔAICc greater than 2) indicate that there exists evidence that these models are different. Therefore, we produced averaged parameter estimates of these selected models (ΔAICc < 2) and we calculated the relative importance of each variable of the models. The *dredge* function was also used to calculate the adjusted R-squared for all the candidate models. The *model.avg* function was used to produce averaged parameter estimates from these sets of selected models and this function also calculated the relative importance of each explanatory variable. Furthermore, we also estimated the 95% confidence intervals (CI) of each parameter through the *summary* function in R. We assessed the significance of each parameter based on whether the 95% CI overlapped zero. The relative importance was calculated as the sum of Akaike weights (AICcW) across all the selected models.

We also calculated between- and within-individual variability of the best selected models. Between-individual variation refers to the classic phenomenon of individual differences in a variable, and within-individual variation refers to variability from occasion to occasion within an individual. We estimated these variances using the *lmer* function in R package "lme4" (Bates et al., 2015). We produced the box-and-whisker plots of the log-transformed dependent variable by groups of explanatory variable using the *ggplot* function in R package "ggplot2" (Wickham, 2016).

Permission for this study was granted through the Commission on Ethics in Animal Experimentation of the University of Jaén, the Consejería de Agricultura, Pesca y Medio Ambiente (Junta de Andalucía), and the Ministry of Economy and Competivity of the Spanish Government.

3. Results

The clinical outcome and evolution of the infested ibexes showed different trends throughout the infestation period. Three of the 25 infested ibexes developed mange lesions on less than 50% of their body surface, whereas 16 progressed to severe stages of the disease (i.e., lesions covering over 50% of the skin surface). Conversely, four of the infested ibexes showed a reduction of the skin lesions during the infestation period, two of them recovering completely. Two of the infested ibexes died during the infestation, with *Corynebacterium pseudotuberculosis* being the identified causative agent (caseous lymphadenitis).

Descriptive statistics for the raw data can be seen in Table 1 and values of 11-k (log-transformed) grouped by the predictor variables are represented in Fig. 1.

The dynamics of the status of the disease (clinic) was not significantly influenced by time post infection (Friedman's chi-squared = 1.8; df = 1; *p*-value = 0.1797).

Model averaging indicated that the most parsimonious model included host age, time (e.g., days post-infection), and clinic (e.g., mange status), which explained 76.6% of the variance of the faecal 11-k concentration. It was followed by a model including time and clinic, which explained 71.83% of variance of the faecal 11-k concentration (Table 2). Components of the selected model for 11-k are described in Table 3.

Male and female ibexes showed similar values of faecal 11-k, but the concentration of this metabolite tended to decrease (although not significantly) with increasing host age (Table 1). Overall, faecal 11-k increased through time, parallel to the development of the disease, reaching values 70 times higher than those reported in free-ranging and healthy animals (Molina-García et al., 2018).

Inter-individual differences accounted for 23.4% of the variance of 11-k concentration.

4. Discussion

Previous studies on the effect of sarcoptic mange on the hematology and serum chemistry of Iberian ibex (see for instance Pérez et al., 2015) lack data on serum cortisol levels as a stress index. Moreover, they involved invasive methods for sample collection (e.g., physical restraint and blood collection) which could favour misinterpretation of results. This is the first study showing the link between the level of sarcoptic mange infection and the stress response measured here by faecal concentration of a cortisol metabolite.

Mange prevalence can reach higher values in female ibex, but severity of sarcoptic mange is male-biased in Iberian ibex (López-Olvera et al., 2015, Castro et al., 2016), particularly during the rutting season (León-Vizcaíno et al., 1999). This could be explained, at least partially, by the immunosuppressive effect of testosterone (Klein, 2004). Sex hormones, reproductive status, and pathogen load all affect stress. Together with stress, these factors can modulate the immune system and affect disease incidence (Cizauskas et al., 2015). Older scabietic free-ranging ibexes tend to harbour lower mite densities, presumably due to previous contacts with the parasite and the consequent development of resistance (Castro et al., 2016). This could explain the lower values of 11-k in the older animals we reported. In our study, host sex and age alone did not seem to have a significant effect on the response variable, but competing models including their additive interaction with time (Table 2) suggest differences in the timing of the stress-induced response (e.g., disease dynamics) due to these intrinsic factors. Nevertheless, further studies are needed to characterize faecal 11-k in infested animals during the early phases of mange infections in order to assess the timing of the immune response caused by the disease (Pence and Ueckermann, 2002) and the adrenocortical activation. In other words, the nature of the action or actions (e.g., permissive, suppressive, stimulating or preparative) (Sapolsky et al., 2000) due to the GC release during the curse of sarcoptic mange infection needs to be fully addressed.

The capture of wild animals in their natural environment involves an extreme additive effect of both physical and psychological stressors (Villiers et al., 1995; Griffin and Thomson, 1998). Moreover, the activity of burrowing mites produces pruritus, among other effects in parasitized hosts, which is considered an important stressor. We must take into account that sarcoptic mange often leads to secondary infestations, loss of weight, and anaemia and also affects internal organs (Pérez et al., 2015; Espinosa et al., 2017b), which can exacerbate the effect of the parasites, or even a stress response linked to a change in the level of body reserves (Groscolas and Robin, 2001). Martin et al. (2018) reported increased metabolic requirements in scabietic wombats (*Vombatus ursinus*) compared with healthy ones, but infested animals were unable to compensate this as they spend most of their time inactive, leading to the depletion of their fat stores.

Moreover, scabietic ibexes worsen their reproductive fitness (Sarasa

Table 1
Descriptive statistics of 11-k by groups. From left to right: Mean, standard deviation (SD), minimum value (Min), first quartile (Q_1), median, third quartile (Q_3) and maximum value (Max). The groups are (from top to bottom): host sex (Sex), time post-infection (Time), mange status (Clinic), and host age (Age).

	Response		11-k						
Groups		N	mean	SD	Min.	Q1	median	Q3	Max.
Sex	Male	22	193.57	124.04	53.10	94.90	155.40	224.84	449.36
	Female	17	198.68	101.03	68.17	103.78	218.94	275.41	359.35
Time	103 days	39	147.66	75.39	53.10	89.45	103.98	220.33	275.13
	131 days	37	238.68	125.96	68.17	136.32	218.94	342.03	449.36
Clinic	Healthy	16	88.47	22.84	53.10	75.84	85.385	106.80	118.09
	Mild	5	154.34	57.15	79.69	106.50	157.60	210.60	224.84
	Severe	16	266.34	113.47	80.49	179.21	275.41	350.69	449.36
Age	≤2 years	14	219.89	80.85	103.57	160.65	219.89	275.13	359.35
	3–5 years	18	193.45	135.14	53.10	90.81	135.32	269.89	449.36
	≥6 years	5	140.91	99.02	79.69	80.49	81.32	154.55	308.50

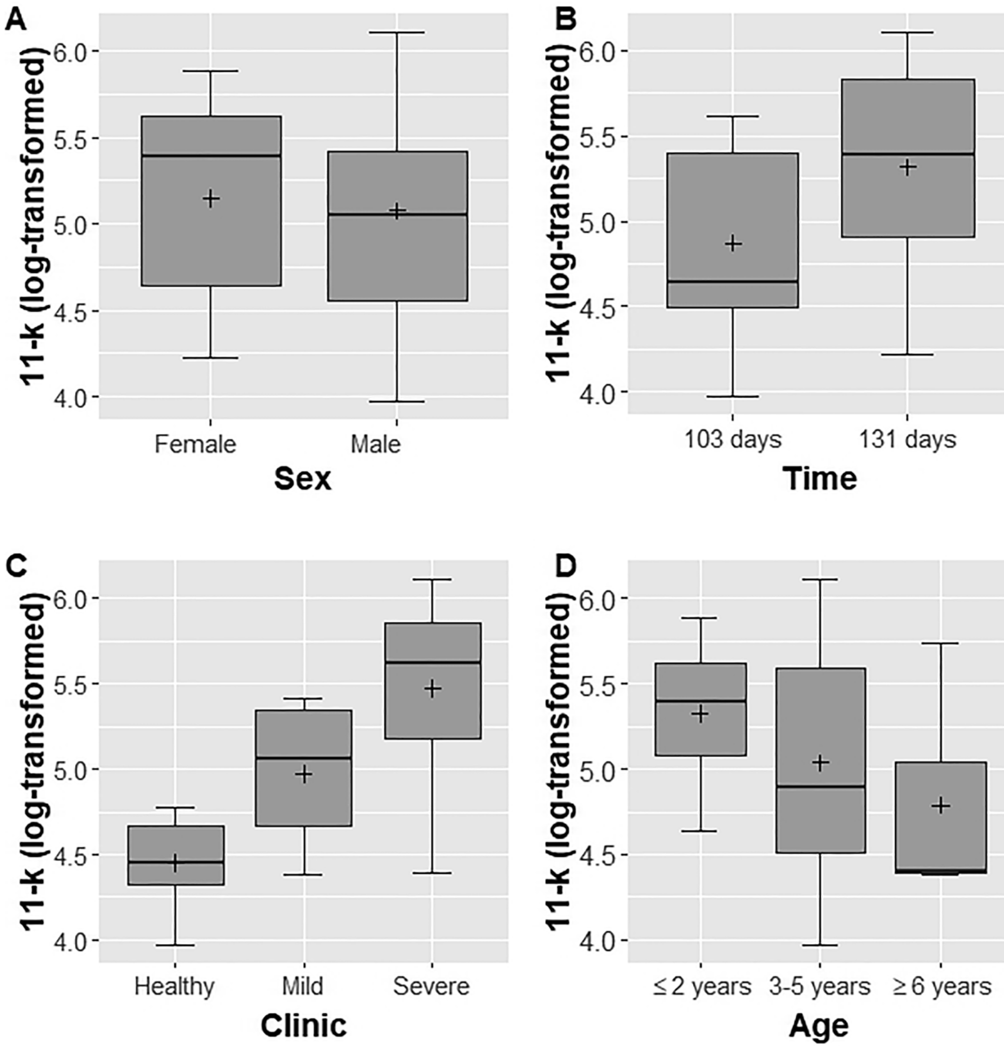

Fig. 1. Effect on 11-k (log-transformed) of: host sex (A); time post-infection (B); clinic (mange status) (C) (0: healthy (control), 1: mild stage, and 2: severe stage); and host age (D). Box-and-whisker plots represent the data: the top and bottom of the boxes represent the first and third quartiles, respectively; the line across the box represents the median, and the cross represents the mean. The vertical solid lines indicate upper and lower limits.

Table 2
Linear mixed model selection for the effects of: host sex (Sex), time post-infection (Time), mange status (Clinic), and host age (Age) on 11-k in Iberian ibex. R^2:adjusted R-squared; AICc: Akaike's Information Criterion corrected for small sample size; ΔAICc: difference of AICc between the model and the most parsimonious model; AICcW: Akaike's weight of the model.

Biological model	R^2	AICc	ΔAICc	AICcW
Age + Time + Clinic	0.7662	46.10	0.00	0.47
Time + Clinic	0.7183	46.78	0.69	0.33
Age + Time + Clinic + Sex	0.7715	48.99	2.89	0.11
Time + Clinic + Sex	0.7186	49.86	3.76	0.07
Time + Sex + Age	0.5649	53.88	7.79	0.01
Age + Clinic	0.6085	54.21	8.11	0.01
(Null model)	0.0318	70.63	24.53	0.00

Table 3
Model averaged standardized coefficients of the linear mixed models for 11-k in Iberian ibex (based only on the two first models summarized in Table 2). Parameters are: host age (Age), Clinic1: mange status = mild, Clinic2: mange status = severe, and time post-infection (Time). β: estimated coefficient; SE: unconditional standard error; 95% CI: confidence intervals at 95% calculated using ± 1.96 SE; w_{rp}: relative importance of overall predictor.

Parameter	β	SE	95% CI		w_{rp}
			2.5%	97.5%	
Age	−0.1222	0.1333	−0.4425	0.0249	0.59
Clinic1	0.4763	0.1483	0.1589	0.7936	1.00
Clinic2	0.8262	0.1463	0.5133	1.1389	1.00
Time	0.3765	0.1126	0.1350	0.6180	1.00

et al., 2011; Espinosa et al., 2017a) and this could be favoured by increasing cortisol metabolite levels, which affects hormone release (Dobson and Smith, 2000; Sheriff et al., 2009). Further research is needed to assess the role of this GC in the development of host skin lesions (epidermic atrophy, hyperkeratosis, increasing dermal fragility, peripheric vasoconstriction, etc.).

Although GCs are helpful during acute stress events they become problematic when elevated chronically (Dhabhar and McEwen, 1997). Chronic increases in GCs can cause, for example, metabolic disruption, reproductive suppression, immunosuppression and immunomodulation, as well as increased susceptibility to disease (Khansari et al., 1999; Cohen et al., 2012; Martin, 2009; Dhabhar, 2014). It is known that in mammals prolonged elevation of cortisol inhibits the production and activity of T-lymphocytes among other important components of the immune response (Sapolsky et al., 2000). This modulation of the immune response could even be adaptive if it allows relocation of resources for preventing immunopathological damage or for other critical processes (Boonstra, 2013). Recent studies (see Cuervo et al., 2018) suggest that chronic stress can enhance innate defences.

Additional research is still needed in order to address seasonal patterns of faecal GCs and the effect of other intrinsic factors, such as the host's genotype, reproductive and immunological status, as well as host behaviour (Cizauskas et al., 2015; Pirotta et al., 2018) on the ibex response to the stressor effect of sarcoptic mange.

The individual response to manipulation (e.g., capture, transportation, physical immobilization for experimental infestation and samples collection) and housing would also deserve particular attention.

Taking into account the immunosuppressive effects of cortisol, the results obtained in this study could help us to better understand the difficulty in acquiring tolerance or resistance to sarcoptic mange by ibexes, and may contribute to forming the scientific basis for managing the disease in wild populations focussed on the capture and treatment of selected individuals (Pence and Ueckermann, 2002). Finally, the method used in this study promises to become a reliable and versatile tool for non-invasive monitoring of stress in wild and naturally-infested animals in order to implement appropriate measures to mitigate the disease as well as the effects of the associated stress response.

5. Conclusion

The impact of sarcoptic mange on ibexes is complex. The mites' activity results in damage to the host's skin and internal organs, favours secondary infestations and loss of the host's body condition and reproductive fitness. Moreover, the disease is an important stressor and through the chronic release of glucocorticoids, which have an immunosuppressive effect, could compromise the immune response of scabietic hosts. Our results evidenced inter-individual differences in the stress response of experimentally-infested ibexes.

Conflict of interest statement

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.05.007.

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