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Steroid hormone related male biased parasitism in chamois, *Rupicapra rupicapra rupicapra*

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Abstract

Parasites are linked with their host in a trophic interaction with implications for both hosts and parasites. Interaction stretches from the host's immune response to the structuring of communities and the evolution of biodiversity. As in many species sex determines life history strategy, response to parasites may be sex-specific. Males of vertebrate species tend to exhibit higher rates of parasites than females. Sex-associated hormones may influence immunocompetence and are hypothesised to lead to this bias. In a field study, we tested the prediction of male biased parasitism (MBP) in free ranging chamois (*Rupicapra rupicapra rupicapra*), which are infested intensely by gastrointestinal and lung helminths. We further investigated sex differences in faecal androgen (testosterone and epiandrosterone), cortisol and oestrogen metabolites using enzyme immunoassays (EIA) to evaluate the impact of these hormones on sex dependent parasite susceptibility. Non-invasive methods were used and the study was conducted throughout a year to detect seasonal patterns. Hormone levels and parasite counts varied significantly throughout the year. Male chamois had a higher output of gastrointestinal eggs and lungworm larvae when compared to females. The hypothesis of MBP originating in sex related hormone levels was confirmed for the elevated output of lungworm larvae, but not for the gastrointestinal nematodes. The faecal output of lungworm larvae was significantly correlated with androgen and cortisol metabolite levels. Our study shows that sex differences in steroid levels play an important role to explain MBP, although they alone cannot fully explain the phenomenon.
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Keywords: Gastrointestinal and lung helminths; Male biased parasitism; Endocrine–immune interaction; Non-invasive methods; Seasonality; Chamois

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1. Introduction

Chamois (*Rupicapra rupicapra rupicapra*) population sizes are primarily limited through density-independent extrinsic weather factors during winter and early spring, when food is limited and climate is harsh; they are only secondarily affected by density-dependent crowding factors like social stress, competition for resources or parasite mediated disease transmission (Crampe et al., 2002; Boschi and Nievergelt, 2003). Endoparasitic infections (gastrointestinal and lung helminths) along with some ectoparasitic infections (mange) and microparasitic diseases (ecthyma contagiosum, infectious keratoconjunctivitis, pneumonia) are the most commonly observed diseases in wild chamois populations (Boch and Schneidawind, 1988; Degiorgis et al., 2000; Rizzoli and Rosà, 2002).

In ungulate populations including chamois there is a trend for the adult sex ratio to be shifted towards females (Owen-Smith, 1993; Jorgenson et al., 1997; Loison et al., 1999a,b). Female bias in adult sex ratio is positively correlated with the degree of male bias in sexual size dimorphism (SSD) (Promislov, 1992). The evolution of SSD is associated with the evolution of male biased mortality linked to greater susceptibility to infection by parasites (Moore and Wilson, 2002). The male biased parasitism (MBP) is most pronounced in polygynous species where male–male competition for mates is most severe. A number of comparative analyses have examined sex biased parasitism (SBP) in free ranging and naturally infected populations and these studies described a small but highly significant tendency for male mammals (prevalence and intensity) and birds (prevalence only) to suffer more from nematode infections than their female counterparts (Poulin, 1996).

There are a number of biological mechanisms potentially capable of leading to SBP situations. They are often divided into ecological and physiological mechanisms (Zuk and Mc Kean, 1996). Ecological mechanisms include sex differences in behaviour, habitat choice, diet composition and body size. Possible physiological explanations for sex biases may result from direct or indirect effects of steroid hormones on components of the immune system and/or on parasite growth and development. According to literature, there is overwhelming evidence that sex-associated hormones not only affect sexual differentiation and reproduction, but also influence the immune system. Androgens, oestrogens and various hormones associated with pregnancy have all been implicated as possible mediators of immunocompetence affecting an organism's overall ability to avoid the harmful effects of parasites (Grossman, 1985; Alexander and Stimson, 1988; Olsen and Kovacs, 1996; Klein, 2000). Testosterone has been shown to be a potent suppressor of both the cellular and humoral components of the immune system. The resulting sex related difference in immunocompetence could be the cause for male biased parasitic disease induced mortality.

Determination of the health status in wild animals, a key tool for management, is often difficult to achieve where animals inhabit remote environments, and where access to samples is limited due to legislative restrictions (i.e. hunting laws). Parasitological examination of faecal samples allows a sanitary survey without handling animals. Non-invasive methods to assess the endocrine status of wild animals are well established (Schwarzenberger et al., 1996; Touma and Palme, 2005). An insight into the complex interactions between the endocrine status of the host, its parasites and the environment is possible by collection of repeated faecal samples. Until now, only faecal cortisol metabolites have been measured previously in chamois (Thaller et al., 2004). The aim of this study was to investigate the relationship between sex related endoparasitic burden and faecal steroid hormone levels in free-ranging chamois throughout a year in the national park Hohe Tauern in Austria, the largest protected area in the European Alps.

2. Materials and methods

2.1. Study area

The study area was situated in the national park Hohe Tauern, Austria (47°06′N, 12°29′E) over an elevation range 1200–2800 m. It incorporates wildlife management areas in three provinces Tirol (Gschlösstäler with approximately 4800 ha and 40 chamois), Salzburg (Kötschach- und Anlauftal with approximately 8200 ha and 350 chamois) and Carinthia (regions around Mallnitz, Malta and Oberes Mölltal with approximately 21 500 ha and 1000 chamois).

Density in chamois varied from <1 to 16 animals/100 ha (2001 and 2004 censuses of park rangers; Slotta-Bachmayr, unpublished data). The study area is also used by Alpine ibex (*Capra ibex ibex*), red deer (*Cervus elaphus hippelaphus*) and roe deer (*Capreolus capreolus capreolus*). Most of the area is situated in the alpine zone above the timberline. Within the protected area, hunting has been prohibited since 2001 except for scientific needs. However, chamois can be hunted outside the reserve during the fall hunting season.

2.2. Sampling

From July 2003 to August 2004, experienced park rangers regularly collected faecal samples in the field. Chamois were observed with binoculars or telescope and categorised by age and sex. Age in chamois can be determined by counting the annual growth rings on the horns and sex is recognised since males in general are slightly heavier, taller and have thicker and more crooked horns than females (Habermehl, 1985). Following observed defecation fresh faeces of approximately 20 g were collected. At each sampling site the age and sex of the chamois, which voided the faeces, as well as the localisation and consistency of the faeces were recorded. Although experienced park rangers carried out the observations, the sex of the animal was not always obvious: of the 141 samples of chamois, sex could not be determined in 43 (30.5%) animals. The park rangers visited different sectors in their assigned territory to collect samples from different individuals over time. As samples were collected over a large area and time frame we assume that each sample defines an individual and that our study includes samples collected from approximately 10% of the total chamois population. In addition to the faecal samples collected after observed defecation, samples from hunted animals ($n = 29$) were taken from the rectum.

Faecal pellets were homogenised immediately after collection. The larger part of the sample was stored at 4 °C until the parasitological investigations were performed at the field station; a smaller part was stored at −20 °C for subsequent steroid metabolite analysis. In order to link seasonal fluctuations of hormone levels and parasitic output four groups correlating to the alpine climate and the life cycle of chamois were defined: January to April (alpine winter with snow cover); May to June (birth season, spring); July to August (alpine summer); September to December (rut season, autumn).

2.3. Parasitological investigations

In order to identify different eggs of gastrointestinal nematodes, cestodes and trematodes, the combined sedimentation–flotation method (CSFM) according to Eckert et al. (1992) with zinc sulphate solution (Gatt-Koller, Absam, Austria; specific gravity of 1.32 at 20 °C) was used. Based on morphology and size, helminth eggs were distinguished under light-optical microscope at 100× and 400× magnifications (Thienpont et al., 1986; Mehlhorn et al., 1993; Rommel et al., 2000). The egg output of gastrointestinal nematodes was classified semi-quantitatively at 100× magnification (Table 1). To quantify the number of eggs per gram (epg) of faeces, the McMaster method described by Kaufmann (1996) was applied. Only eggs of gastrointestinal nematodes were counted without differentiating the species. A simplified coding key was defined for easier recording (Table 1). The Baermann–Wetzel method was applied to isolate lungworm larvae (Eckert et al., 1992). The first-stage larvae were identified by their size and tail morphology at 200× and 400× magnification (Mehlhorn et al., 1993). A scale was created to semi-quantitatively determine the larval output at 50× magnification (Table 1).

2.4. Endocrine investigations

Faecal hormone metabolites including immunoreactive androgen, cortisol and oestrogen metabolites were analysed by enzyme immunoassays (EIA) using established methods verified in a wide range of animal species (Schwarzenberger et al., 1996; Touma and Palme, 2005). Hormone extraction from faecal samples was performed as described previously (Palme and Möstl, 1997); faeces (0.5 g) was mixed with 0.5 ml of water and 4 ml of methanol (80%), and vortexed for 30 min. After centrifugation the supernatant was diluted with assay puffer (1:10) and extracts were analysed by EIA using group-specific antibodies. These antibodies show considerable cross-reactivity to hormone metabolites with similar functional groups in the steroid molecule. As these assays concurrently detect several steroid metabolites their use has been proven to be advantageous over rather specific commercially available assays (Schwarzenberger et al., 1997; Touma and

Palme, 2005). The androgen assays used in this study included 17-oxo-androstanes (5α-androstane-3β-ol, 17-one 3-HS:BSA; trivial name: epiandrosterone) and 17β-OH-androstanes (4-androstene-17β-ol, 3-one 3CMO:BSA; trivial name: testosterone; Palme and Möstl, 1993). Cortisol metabolites were measured using an antibody against 11,17-dioxoandrostanes (immunogen: 11-oxoetiocholanolon-3-HS:BSA; Palme and Möstl, 1997) and total oestrogens were analysed using an antibody against estradiol-17β-OH 17-HS:BSA; Schwarzenberger et al., 2000). Quality control including intra- and interassay variability, coefficient of variation, and faecal extracts that were dilutable parallel to the standard curve were within the range of comparable enzyme- or radioimmunoassays.

2.5. Statistical analysis

For the calculation of the overall and seasonal prevalence of faecal parasites samples from determined and undetermined sexes were used (Table 1), whereas for the more detailed analyses of the association between hormones, sex and parasites only the samples of chamois with determined sex were used. The preva-lence, defined as the proportion of parasite positive samples divided by the total number of samples examined, was calculated for gastrointestinal worm eggs, lungworm larvae as well as for the different parasite species detected. Spearman rank correlation was used to compare the results of the CSFM and the McMaster technique. The Fisher's exact test (FET) was applied to study variation in egg and larval output during the cold and the warm seasons, for which the period from September to April was summarised as cold season, and the period from June to August as warm season. The following analyses considered only samples collected from samples of known sex. The Chi-square test (χ^2) and Kruskal–Wallis one-way analysis of variance (ANOVA) – depending on the format of the outcome – were used to assess sex and seasonal differences in parasitic burden. A Poisson regression model was used to test the influence of sex, season and age on egg count data (epg). All steroid metabolite values were log-transformed to obtain normally distributed data, and Spearmann rank correlations for the different steroid metabolites were calculated. Mean steroid levels for all groups were reported with the 95% confidence intervals. Seasonal and sex influences on the steroid levels were studied

Table 1
Coding and descriptive statistics of gastrointestinal egg and lungworm larvae output

Parasites, method	Code	%	Simplified categories	%
Gastrointestinal nematodes, CSFM				
No eggs	−	6	Low output (<10 eggs)	52
Less than 10 eggs on the whole slide	+	46		
Fields of vision with isolated eggs	++	26	High output (>10 eggs)	48
Continuously eggs in the fields of vision	+++	13		
Fields of vision with many eggs	++++	9		
Eggs per gram (epg) of gastrointestinal nematodes, McMaster				
<50 eggs per gram	−/+	34	Low output (≤50 eggs)	63
50 eggs per gram	+	29		
100 eggs per gram	++	12	High output (>50 eggs)	37
150 eggs per gram	+++	11		
≥200 eggs per gram	++++	14		
Lungworm larvae, Baermann				
No larvae	−	14	Low output (≤5 larvae)	41
Less than 5 larvae	+	27		
Isolated accumulations of larvae	++	25	High output (>5 larvae)	58
Fields of vision mainly with larvae	+++	22		
Fields of vision with high numbers of larvae	++++	11		

Parasitological examination included faecal samples from 141 chamois collected between July 2003 and August 2004 in the national park Hohe Tauern. Classifications and a corresponding code were created for the three different methods (CSFM, McMaster, Baermann) used and the involved parasites. Simplified categories were used for further statistical analysis. Results are reported as prevalence (%).

using one and two-way ANOVA tests with the Bonferroni (all pair wise) multiple comparison correction. To test for possible sex biased parasitism originating in different steroid hormone levels, a three-way ANOVA model was applied. Here, the means of steroid metabolite levels (response variable) were set in relation to parasitic output, different sex and seasons (factors in the model). The most reliable data were available from the subset of 12 hunted female and 17 hunted male chamois. All statistical analyses were repeated as described for the full set data and the results were compared. All statistical procedures were performed with the NCSS 2001 software (Number Cruncher Statistical Systems. Kaysville, UT, USA) and Microsoft Excel. The overall level of statistical significance was set to $p < 0.05$.

3. Results

3.1. General

The study included 141 samples of chamois of which 21% were collected from hunted animals and 79% were obtained following observation of the animals in the field. Fifty-three samples were determined to originate from females and 45 from males; in 43 samples the sex could not be reliably determined. The age of the animals was estimated in 76 cases with a median of 4 years (95% confidence interval: 3–5 years).

3.2. Parasitology

The prevalence of gastrointestinal nematode eggs was very high with 94%. Most frequently eggs from the family of Trichostrongylidae were detected while cestode and trematode eggs were nearly absent (for details see Table 2). The mean faecal epg was 92 with a maximum of 600. Correlation between the McMaster method and CSFM was high ($r = 0.83$). Larvae of lung nematodes were present in 86% of the samples. In most instances (85%) Protostrongylidae (small lungworms) were detected. *Dictyocaulus* sp. (big lungworms) had a prevalence of 8% (Table 2). A marked seasonality in parasitological output was noted. A significantly higher nematode egg output was determined during the warm season as compared to the cold season (FET, $p = 0.001$), whereas larval output of lungworms was higher during the cold than during the warm season (FET, $p = 0.009$).

Considering the results of the McMaster technique, a significant difference in epg between samples from

Table 2
Prevalence and differentiation of phyla, families and genera of faecal parasites in chamois ($n = 141$) collected between July 2003 and August 2004 in the national park Hohe Tauern

| Phylum	Family	Genus	Prevalence (%)
Gastrointestinal nematodes, cestodes and trematodes			
Nematoda	Trichostrongylidae	*Ostertagia* sp. (*Telodorsagia* sp.)	74
		Trichostrongylus sp.	44
		Marshallagia sp.	26
		Haemonchus sp.	24
		Cooperia sp.	9
	Chabertiidae	*Chabertia* sp.	25
		Oesophagostomum sp.	4
	Trichuridae	*Trichuris* sp.	14
		Capillaria sp.	1
Cestoda	Anaplocephalidae	*Moniezia* sp.	1
		Toxocara sp.	3
Trematoda	Dicrocoeliidae	*Dicrocoelium* sp.	1
Lungworms			
Nematoda	Protostrongylidae	*Neostrongylus* sp.	61
		Muellerius sp.	58
		Protostrongylus sp.	23
		Cysocaulus sp.	5
	Dictyocaulidae	*Dictyocaulus* sp.	8
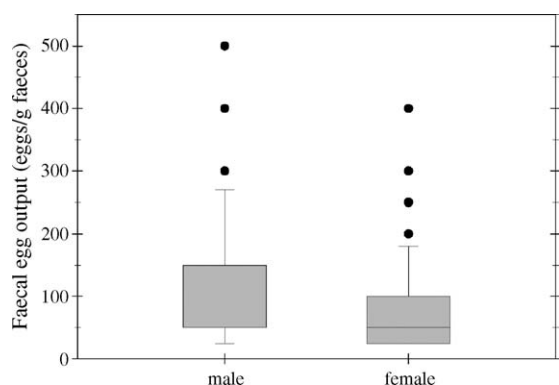
Fig. 1. Sex differences in the output (epg) of gastrointestinal nematodes in faecal samples of chamois ($n = 98$). The median epg value in both sexes was 50, but results between males ($n = 45$) (mean rank = 57 epg) and females ($n = 53$) (mean rank = 43 epg) were significantly different (one-way ANOVA, $p = 0.013$).

male and female chamois was evident (ANOVA on ranks, $p = 0.013$, Fig. 1) even though the median epg value was identical for both sexes. Based on the CSFM and Baermann results the sex differences were neither significant for the nematode eggs (χ^2, $p = 0.156$) nor for the lungworm larvae (χ^2, $p = 0.053$), although the latter result indicated a trend. Overall, the tendency for males to harbour more parasites was apparent: 26 out of 45 male samples (57.8%) had a high output of nematode eggs, whereas the comparable result in females was only 23 out of 53 (43.4%). For lungworm larvae, results for the male samples were 30 out of 45 (66.7%), and for the females 25 out of 53 (47.2%).

Sex ($p = 0.0035$) and season ($p < 0.0001$) were significant factors in the Poisson regression model on faecal epg count. Male samples showed a 1.6 times higher risk of higher epg count when compared to females. The seasonal effect was even more pronounced than the sex effect, with a 2.1 times higher risk for high epg values during the warm season when compared to the cold season. The risk for higher epg with increasing age was not significant ($p = 0.075$) in this model.

3.3. Endocrinology

Fluctuations in hormone levels were detectable throughout the year and results are summarised in Fig. 2.

3.3.1. Epiandrosterone like metabolites (17-oxo-andostanes)

Fluctuations in hormone levels were detectable throughout the year, and the low values observed during summer were significantly different from those in the other seasons (Fig. 2). The mean value of the male samples (1997 ng/g; 1316–3028) differed significantly (ANOVA, $p < 0.001$) from those of the females (702 ng/g, 478–1031). These seasonal variations were significant for both sexes (ANOVA, $p = 0.005$): lowest values for male (763 ng/g, 379–1534) and female (250 ng/g, 115–534) samples were determined during summer and a sharp increase occurred around the autumn rut in males (mean = 3573 ng/g, 1776–6783), but not in females (mean = 398 ng/g; 148–1069).

3.3.2. Testosterone like metabolites (17β-OH-androstanes)

Again, mean values of male samples (225 ng/g, 144–352) were significantly higher (ANOVA, $p < 0.001$) than those of females (88 ng/g, 58–133). Seasonality in hormone levels was highly significant (ANOVA, $p < 0.001$). During autumn and winter the values were elevated with males showing the highest concentrations (Fig. 2). Values in summer were significantly different from those of the other seasons.

3.3.3. Cortisol like metabolites (11,17-dioxoandrostanes)

No significant sex related differences were detected (Fig. 2; mean of male and female samples = 1204 ng/g, 918–1579), however, cortisol metabolite excretion revealed a significant seasonal pattern (ANOVA, $p = 0.01$) with maximum levels observed during winter and minimum levels during summer; values in summer were significantly different from those in autumn and winter. Mean values during winter were 2954 ng/g (1059–8240) for males and 1446 ng/g (771–2710) for females.

3.3.4. Total oestrogens

No significant sex differences were noted (mean of male and female samples = 45 ng/g, 36–57), but seasonality in hormone levels was highly significant (ANOVA, $p < 0.001$). Highest values were determined for the winter (mean = 98 ng/g, 64–149) and
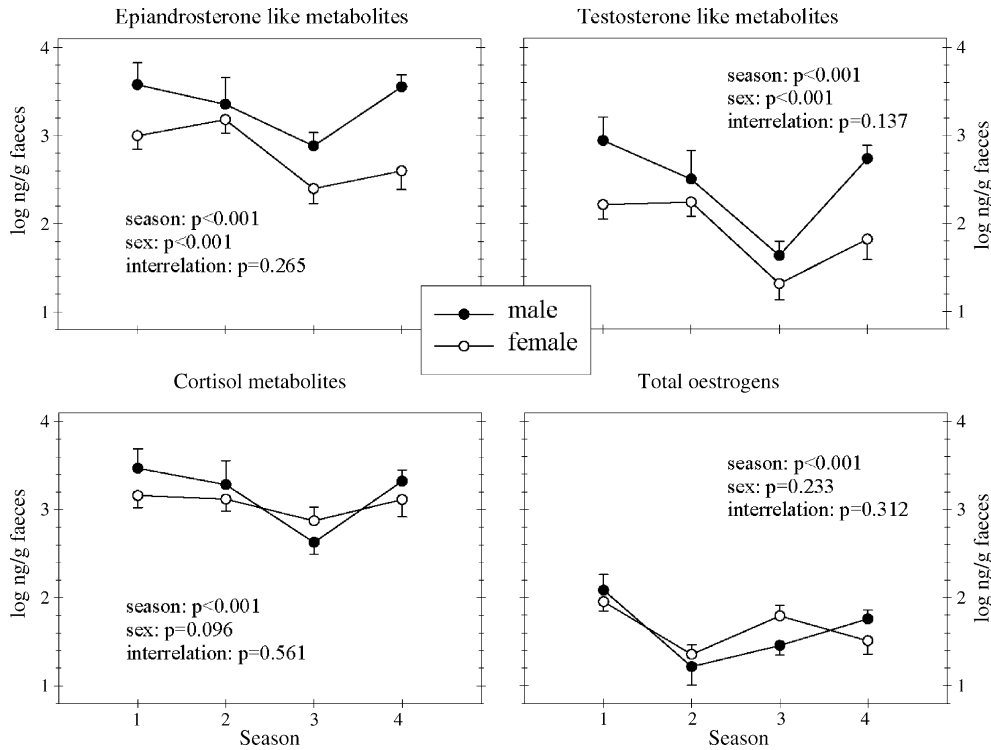
Fig. 2. Seasonal changes in the levels of four log-transferred steroid metabolites measured in faecal samples from 45 male and 53 female chamois collected between July 2003 and August 2004 in the national park Hohe Tauern. Seasons were defined according to the life cycle of chamois: (1) January–April, (2) May–June, (3) July–August, (4) September–December (means derived from a two-way ANOVA).

values in winter were significantly different from those in spring and summer.

The correlation between epiandrosterone and testosterone metabolites was high ($r = 0.83$). Cortisol metabolites revealed a lower correlation with epiandrosterone ($r = 0.59$) and testosterone metabolites ($r = 0.57$) while oestrogen measurements showed only low correlations with androgen ($r = 0.17$) and cortisol metabolites ($r = 0.25$).

3.4. Relationship between parasites, sex, season and steroid metabolites

The output of lungworm larvae was significantly correlated with epiandrosterone (ANOVA, $p = 0.031$), testosterone (ANOVA, $p = 0.032$) and cortisol (ANOVA, $p = 0.026$) assays (Table 3). Males with high androgen levels (epiandrosterone: ANOVA, $p = 0.005$; testosterone: ANOVA, $p = 0.007$) showed a higher larval output when compared to females.

Cortisol metabolites per se were not significantly influenced by sex, however, larval output was significantly correlated to cortisol metabolites (ANOVA, $p = 0.012$), and males with high larval output showed elevated cortisol metabolites.

The investigation of gastrointestinal nematodes revealed no significant relationship between egg output, sex and steroid levels. Considering the semi-quantitative parasitological results, only oestrogen levels (ANOVA, $p = 0.017$) had a significant influence on egg output in spring. Independent of the sex, samples of chamois with high egg output had lower oestrogen levels than those with low egg output (Table 3).

3.5. Subset of hunted animals

The hunted animals ($n = 29$) were collected between August and November 2003, and one chamois was hunted in July 2004. The median age was 3 years (2–5). Sex differences in parasitic output were significant.

Table 3
A three-way ANOVA model with interaction terms for the different parasitological investigations (Baermann, McMaster and CSFM), sexes and seasons with the four log-transformed faecal steroid metabolites in faecal samples from chamois ($n = 98$) of identified sex

Corresponding variables	Epiandrosterone like metabolites	Testosterone like metabolites	Cortisol like metabolites	Total estrogens
Lungworm larvae				
Larval output	0.031	0.032	0.026	n.s.
Sex	0.005	0.007	n.s.	n.s.
Season	0.006	<0.001	0.027	0.008
Larval output and sex	0.037	0.016	0.012	n.s.
Epg gastrointestinal nematodes				
Epg	n.s.	n.s.	n.s.	n.s.
Sex	0.005	0.005	n.s.	n.s.
Season	n.s.	n.s.	n.s.	0.012
Season and sex	0.036	0.004	0.031	n.s.
Gastrointestinal nematodes				
Egg output	n.s.	n.s.	n.s.	n.s.
Sex	<0.001	0.003	n.s.	n.s.
Season	0.044	n.s.	0.089	0.004
Egg output and season	n.s.		n.s.	0.017

Only the significant two and three-way interactions are shown. n.s. = not significant.

Males showed a higher output of lungworm larvae (FET, $p = 0.03$) and gastrointestinal nematode eggs (epg, FET; $p = 0.02$; CSFM, FET, $p = 0.008$) when compared to females. Means of epiandrosterone metabolites in males (4383 ng/n, 2019–9516) differed significantly (ANOVA, $p < 0.001$) from those of females (265 ng/g, 105–668). Sex differences between testosterone metabolites were also highly significant (ANOVA, $p < 0.001$); the means of males were 707 ng/g (331–1510), whereas those of females were at 38 ng/g (15–94). Cortisol metabolites did not differ significantly between sexes (ANOVA, $p = 0.071$). Nevertheless, males showed higher mean values (2541 ng/g, 1245–5187) than females (896 ng/g, 383–2094) and their variance over the mean was notable. Total oestrogens did not differ significantly between sexes (ANOVA, $p = 0.382$). However, males (61 ng/g, 39–96) had higher means than females (45 ng/g, 28–75).

4. Discussion and conclusion

The purpose of this study was to test the hypothesis of male biased parasitism (MBP) originating in different steroid hormone levels between the sexes in wild chamois. For this purpose, non-invasive methods to assess parasitological and endocrine status

in collected faecal samples were used. In addition, rectal samples from hunted animals were available. Hormone levels and parasite counts varied significantly throughout the year. In general, our results concerning the prevalence of gastrointestinal nematodes, cestodes, trematodes and lungworms concurred with earlier observations (Stefancikova et al., 1999; Broglia et al., 2002). Male biased parasitism was more pronounced for the subset of hunted animals than for the entire sample. A possible explanation for this difference is sex misinterpretation following observation and falsely allocated faecal samples in the field. Another factor that needs to be considered is that the subset samples were collected predominantly around the period of rut when behaviour and androgen levels between sexes differ most significantly.

The hypothesis of MBP was confirmed for the output of lungworm larvae: males with higher levels of androgens and cortisol showed higher larval output. In contrast, sex related differences in gastrointestinal nematode load were noted, but these were not significant. Our results concerning the sex related differences are in agreement with an earlier observation in this species from the Pyrenees (Donat and Ducos de Lahitte, 1989) but are in contrast to results in chamois from the Bauges massif (French Alps), where prevalence estimates and intensities of small

lungworms were found to be higher in females than in males (Nocture et al., 1998). Our study was also concordant with earlier surveys in regard to seasonality in egg and larval output (Donat and Ducos de Lahitte, 1989; Alcouffe et al., 1992; Nocture et al., 1998).

This study shows that both faecal androgen metabolites are appropriate to measure endocrine status in free-ranging male chamois. When comparing the androgen metabolites used in this study, significances were comparable for 17β-OH-androstanes (testosterone) and 17-oxo-androstanes (epiandrosterone). We suggest that in further studies in chamois it is sufficient to measure only one of the androgen metabolites. The observed correlation between androgens and cortisol metabolites in this study is probably caused by cross-reactions with certain androgen metabolites of the etiocholanolon antibody used for cortisol metabolite measurements. Nevertheless, the methodology used for the determination of cortisol metabolites has been shown previously to give reliable results in several ruminant species (Touma and Palme, 2005). Under conditions of chronic stress secretion of gonadotropic hormones may be suppressed (Tilbrook et al., 2000). However, the seasonal correlation found in this study appears to demonstrate that androgen output during the mating season overrides a possible suppressive glucocorticoid effect.

The recorded seasonal changes in androgen metabolites are in agreement with the reported behavioural sexual cycle of chamois. In this species, rut starts at the end of October and lasts until December, depending on population density and the age of females (von Hardenberg et al., 2000). Most ovulations occur in the second half of November. Although the adult buck produces sperm throughout the year, spermatogenesis increases in July to reach its maximum in November. Testis and epididymis weights follow this seasonal pattern with the greatest weights around rut (Geiser, 1980). In this study the highest values of androgen metabolites concur with this heightened activity of the male reproductive organs. These high androgen levels persisted into the winter season. Domestic sheep exhibit a sexual cycle similar to that of chamois. Although ewes have their mating season at the beginning of autumn, the highest testosterone blood levels in rams are reported in autumn and winter (Döcke, 1981). The author postulated that this could be due to the prolonged disposition of bucks to mate with late females. In chamois the latest documented mating occurred at the end of January (Meile and Bubenik, 1979). Additionally, androgen levels – irrespective of sex – were generally elevated during winter. Aside from the trophic effects on the male genital tract, the secondary sexual traits and male behaviour, androgens also have an anabolic effect and exert a variety of physiological effects (Nieschlag and Behre, 2004).

We hypothesise that the faecal cortisol metabolite levels measured in this study represent basal cortisol production rather than stress responses due to disturbances. A clear seasonal pattern of faecal cortisol metabolites with significantly higher concentrations during the September to April period was observed in both sexes of chamois. The elevated cortisol production in the cold season may be one of the multiple adaptations to the extreme extrinsic weather factors. Glucocorticoids exhibit a key role in maintaining homeostasis through their proteolytic and lipolytic effects as well as their stimulation of gluconeogenesis (Matteri et al., 2000). Previously, a similar variation of faecal cortisol metabolites with peak levels during winter was reported in captive chamois (Thaller et al., 2004) and in captive red deer (Huber et al., 2003). In addition, plasma cortisol levels in domestic goats showed a similar seasonal variation (Alila-Johansson et al., 2003). However this effect was not reported in cervid studies which examined cortisol in the plasma (Monfort et al., 1993; Bubenik et al., 1998).

As in previous wild ungulate studies, cortisol metabolites in this study did not differ statistically between females and males (Bubenik et al., 1998; Huber et al., 2003). Nevertheless, the higher values in males during fall and winter are evident. The cortisol metabolite levels may have been affected to some extent by cross-reactions with androgen metabolites in the cortisol metabolite assay used. However, the higher values in males can also be explained with the significant behavioural differences between the sexes. In a polygynous mating system rut is an exhausting time. Males barely eat, cover long distances, chase each other and loose weight (Hamr, 1984; Lovari and Locati, 1991).

The interactions between endocrinology, susceptibility to parasitic infections and sex differences have to be seen in a complex matrix that takes into account the special biology of these mountain ungulates. Considering the ecological and evolutionary history of chamois is important in order to understand patterns of disease

transmission in general and the factors explaining SBP. When considering the output of lungworm larvae, this study demonstrated a significant correlation between male biased parasitism and steroid metabolites (androgens and cortisol). The immunosuppressant effects of these hormones may explain the greater susceptibility of males to infection by parasites and developing disease. A similar observation was made previously in reindeer (*Rangifer tarandus tarandus*; Gaudernack et al., 1984). The stress of the rutting season with elevated glucocorticoid levels is hypothesised to reduce humoral antibodies and to enhance larval output of nematodes in males (Blecha, 2000). In contrast to our results a recently published study in bighorn sheep (*Ovis c. canadensis*; Goldstein et al., 2005) did not find a correlation between faecal lungworm and faecal glucocorticoid levels. Furthermore, the seasonal variation of glucocoticoid levels in this study was in disagreement with our observations.

In contrast to the output of lungworm larvae the male bias in quantitative gastrointestinal nematode output was not significantly correlated with sex differences in steroid levels. This shows that additional factors like season, life history, body size, home range, social factors, diet composition or host genetics affecting immune function need to be considered. Another factor is the difference in life-history trajectories of the sexes. Females gain fitness through longevity and sequential reproductive effort and subsequently need to invest more in immune function. Males, on the other hand, gain fitness by increasing their immediate mating success at the possible cost of longevity (Rolff, 2002).

Body size is hypothesised to contribute to SBP (Arneberg et al., 1998) even though SSD in chamois is not very high when compared to other ungulates. The larger males may ingest dose related more infective larvae when compared to females (Halvorsen and Bye, 1999), and larger animals offer a larger target to parasites due to their bigger intestinal tract. Another explanation is that the maintenance of a larger body is costly in terms of a limiting resource with the result that energy allocation to immune function might be affected (Sheldon and Verhulst, 1996). Sex-based differences in home range correlated to body size (Lindstedt et al., 1986), may be a further contributing factor to the sex biased parasitism as the exposition to parasites in a larger area is higher (Brei and Fish, 2003).

Additionally, social factors may influence immune function and resistance to disease as studies in red deer have shown (Hanlon et al., 1997). The accumulation of individuals known as crowding effect is a risk factor that facilitates parasite transmission (Zander, 1998). During the short summer, female chamois with their young occupy large territories when compared to the solitary or small groups of males (Boschi and Nievergelt, 2003). This sex specific spatial distribution entails different diet compositions that may influence parasitic burden. A study of Cantabrian chamois (*Rupicapra pyrenaica parva*) demonstrated a higher consumption of grass-forb by females when compared to males throughout the year (Pérez-Barberia et al., 1997). It is known in ewes that protein supplementation enhances resistance to infection from gastrointestinal nematodes (Kahn et al., 2003). On the other hand, it has to be considered that during periods of high nutrient demand such as rut in males, pregnancy or lactation in females, immune function may take a lower priority than the reproductive effort (Coop and Kyriazakis, 1999).

Another factor exceeding our investigations is host genetics that may affect parasitic disease resistance. Other authors have suggested that females can discern parasitised males based on secondary sex traits that can be used as indicators of resistance to parasitic disease (Hamilton and Zuk, 1982). This idea was developed further by suggesting that the link between secondary sex traits and parasitism might be mediated by androgens and as a consequence, only males with a high degree of genetic resistance to parasitic disease will be able to produce high levels of androgens (Folstad and Karter, 1992). Additionally, the predicted correlation between the mating system and the difference between male and female susceptibility to parasitic disease needs to be considered (Zuk, 1990). Differences in parasitic infection may be reflected by the intrasexual reproductive competition that increases with the degree of polygyny.

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