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Authors: Madslien, Knut, Stubsjøen, Solveig Marie, Viljugrein, Hildegunn, Ytrehus, Bjørnar, Solberg, Erling Johan, et al.

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Hair Cortisol Concentration and Body Mass in Moose (*Alces alces*) Infested with Deer Keds (*Lipoptena cervi*)

Knut Madslien,[1,7] Solveig Marie Stubsjøen,[1] Hildegunn Viljugrein,[1] Bjørnar Ytrehus,[1,2] Erling Johan Solberg,[2] Lucy Kapronczai,[3] Atle Mysterud,[4] Jacques Godfroid,[5] David M. Janz,[3] and Marc Cattet[6] [1]Norwegian Veterinary Institute, Department of Animal Health, Welfare and Food Safety, PO Box 750 Sentrum, N-0106 Oslo, Norway; [2]Norwegian Institute for Nature Research, PO Box 5685 Torgard, NO-7485 Trondheim, Norway; [3]Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4, Canada; [4]Centre for Ecological and Evolutionary Synthesis, Department of Biology, University of Oslo, PO Box 1066 Blindern, NO-0316 Oslo, Norway; [5]Department of Arctic Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT–the Arctic University of Norway, PO Box 6050 Langnes, NO-9037, Tromsø, Norway; [6]RGL Recovery Wildlife Health & Veterinary Services, 415 Mount Allison Crescent, Saskatoon, Saskatchewan S7H 4A6, Canada; [7]Corresponding author (e-mail: knut.madslien@vetinst.no)

ABSTRACT: The deer ked (*Lipoptena cervi*), a hematophagous ectoparasite of cervids, is currently spreading in Scandinavia, and the moose (*Alces alces*) is its main host. However, little is known about the impact of deer keds on moose. We analyzed the hair cortisol concentration (HCC) from 262 moose harvested in the fall in relation to age class, sex, body mass (BM), and deer ked infestation intensity, and BM in relation to age class, sex, and infestation intensity. We found that HCC decreased with increasing deer ked intensity at low ked intensities, but for the higher levels of ked intensities, there was a positive relationship between HCC and ked intensity. The HCC was higher in males than in females and lower in yearlings than in calves and adults. Our failure to find any association between BM and deer ked intensity suggested a negligible impact of deer ked infestation on moose foraging and metabolism at the level of infestation observed early in the infestation, but did not exclude an effect later in winter. Our findings suggested that moose generally tolerated moderate parasitism by keds. However, the increase in HCC at higher ked intensities suggested that the tolerance strategy could be disrupted with further increases in intensities and consequently may negatively affect animal health and welfare.

Key words: Body mass, chronic stress, deer ked, hair cortisol, moose, parasitism, welfare.

The deer ked (*Lipoptena cervi*) is a hematophagous louse fly (Hippoboscidae) found on moose (*Alces alces*), red deer (*Cervus elaphus*), and other species of deer in Europe, Asia, and North America (Bequaert 1942). In many areas, the swarming keds constitute a major obstacle for human outdoor activities, but the parasite has been regarded as harmless for cervids (Allan 2001; Paakkonen et al. 2012). However, Madslien et al. (2011) reported an outbreak of severe alopecia in moose associated with massive infestation of deer keds in Norway, and Kynkäänniemi et al. (2014) stated that reindeer (*Rangifer tarandus tarandus*) showed signs of distress and pruritus after infestation with keds.

Hair analysis is becoming increasingly popular as a method to examine glucocorticoid (GC) and sex steroid hormone concentrations in wild mammals (Koren et al. 2019). Glucocorticoid concentrations are regarded as physiological indices of stress and the relative condition or health of individuals and populations (Bonier et al. 2009).

We aimed to 1) examine the relationship between infestation intensity of deer keds and hair cortisol concentration (HCC) in moose and 2) test whether there was a negative association between infestation intensity of deer keds and moose body mass (BM). Our hypothesis was that increasing intensities of deer keds results in increased HCC and decreased BM.

Skin samples were collected from 262 moose: 36 calves (16 males/20 females), 92 yearlings (56 males/36 females), and 134 adults (67 males/67 females) in Hedmark and Akershus counties, in southeastern Norway, during the first week of the hunting season (5–12 October 2010). Hunters recorded sex and age class (calf, yearling, adult) of the moose, measured the carcass weight (about 50% of full BM; Wallin et al. 1996) and collected a standardized 20×20-cm skin

TABLE 1. Summary statistics for deer ked (*Lipoptena cervi*) intensity (keds/cm^2), hair cortisol concentration (HCC; pg/mg), and body mass (kg) of moose (*Alces alces*; *n*=262) infested with deer keds in southeastern Norway.

		Ked intensity			HCC			Body mass		
Group	*n*	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Calf	36	0.26	0.052–0.700	0.17	2.04	0.17–9.90	1.96	72	42–97	13
Female	20	0.31	0.065–0.700	0.18	1.57	0.17–3.40	0.87	68	42–88	12
Male	16	0.21	0.052–0.441	0.12	2.63	0.35–9.90	2.71	76	60–97	13
Yearling	92	0.34	0.004–1.405	0.28	1.35	0.14–3.97	0.70	131	75–185	20
Female	36	0.33	0.004–1.321	0.27	1.24	0.14–3.16	0.61	126	92–160	16
Male	56	0.34	0.033–1.405	0.29	1.41	0.19–3.97	0.75	134	75–185	22
Adult	134	0.25	0.007–1.167	0.22	1.78	0.22–9.21	1.43	191	129–320	35
Female	67	0.22	0.024–0.598	0.16	1.4	0.23–8.23	1.06	177	129–271	25
Male	67	0.28	0.007–1.167	0.26	2.09	0.22–9.21	1.68	206	130–320	38
Total	262	0.28	0.004–1.405	0.24	1.66	0.14–9.90	1.34	154	42–320	51

sample from the neck area (Madslien et al. 2012).

In the laboratory, guard hairs for HCC analysis were collected by shaving a skin area of 2×2 cm. The hair samples were stored in paper envelopes at room temperature until analysis. Finally, deer ked infestation intensities were calculated from all skin samples (Madslien et al. 2012). The cortisol analysis was performed at the Toxicology Centre, University of Saskatchewan (Saskatoon, Saskatchewan, Canada). Only guard hairs with the follicles removed were used to determine HCC. Surface contamination was removed by washing hairs with methanol (five 3-min washes). Between 100 and 200 mg of washed and dried hair was ground to a fine powder, using a ball mill, and weighed. Extraction of cortisol from hair and cortisol concentration (pg/mg powdered hair) were determined as described by Macbeth et al. (2010).

Generalized additive modeling with gamma distribution and inverse link was run to analyze the effect of ked intensity on HCC. Ked intensity was transformed (natural log) to normalize and stabilize the variance. Age class, sex, and BM were then added to the baseline model (i.e., forward selection), using the small-sample–corrected Akaike information criterion (Burnham and Anderson 2003). We used generalized linear regression to analyze the effect of age class, sex, and ked intensity on moose BM. Statistical analyses were performed using the R statistical software (R version 2.14.1; R Development Core Team 2019) and the generalized additive modeling was run in the R package mgcv.

Mean ked intensity (mean number of keds/cm^2) in skin samples and HCC varied among sex and age classes (Table 1). The HCC decreased with increasing infestation intensity at low ked intensities, but for the higher levels of ked intensity, there was a positive relationship between HCC and infestation intensity (Fig. 1A). Yearlings had lesser HCC than adults (P=0.003) and males had greater HCC than females (P<0.001; Table 2). When analyzing moose with deer ked intensities greater or equal to the mean, we found a slight increase in HCC with increasing ked intensities (Table 3 and Fig. 2). Variation in BM was best explained by age class and sex (Fig. 1B and Table 4).

Varying results have been found in studies evaluating the relationship between parasite intensity and GCs (Goldstein et al. 2005; Carlsson et al. 2016). We found that males have greater HCC than females and that HCC was positively associated with BM larger than 150 kg (i.e., adults; Fig. 1B). Similarly, Di Francesco et al. (2017) found significantly greater HCCs in muskoxen males than females. Body mass was found to be negatively associated with HCC for BM less than 125

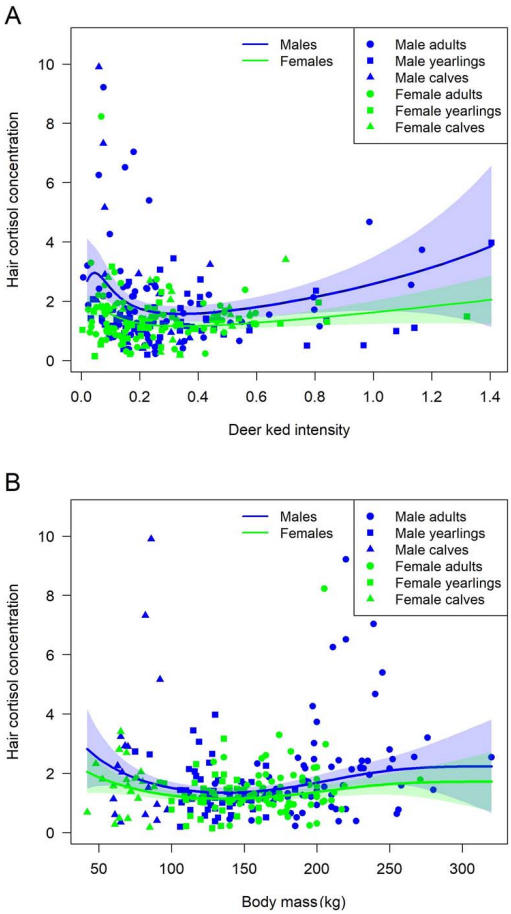

FIGURE 1. Prediction of hair cortisol concentration (HCC; pg/mg) shown as a spline function of deer ked (*Lipoptena cervi*) intensity (keds/cm^2) (A) and body mass (kg) (B) in 262 Norwegian moose (*Alces alces*). Prediction lines together with 95% confidence envelopes are shown for males (blue) and females (green) at the level of (A) overall mean of deer ked intensity and (B) mean body mass of adult females and adult males, respectively. Raw data of deer ked intensity and corresponding HCC are imposed as points of various symbols for the three age classes.

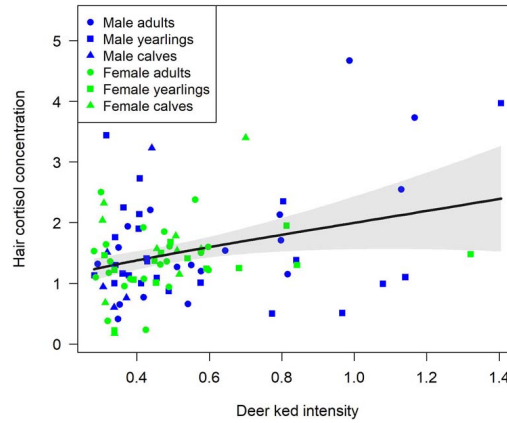

FIGURE 2. Prediction of hair cortisol concentration (HCC; pg/mg) shown as a function of deer ked (*Lipoptena cervi*) intensity (keds/cm^2) for Norwegian moose (*Alces alces*) with above mean infestation intensity (prediction lines together with 95% confidence envelopes). Raw data of deer ked intensity and corresponding HCC are imposed as points of various symbols for the three age classes.

kg (i.e., yearlings; Fig. 1B). This association agrees with Mislan et al. (2016), who reported polar bears in poorer body condition had greater HCC.

According to the cort-fitness hypothesis, high baseline GC concentrations indicate an individual or population in poorer condition and with lower relative fitness (Bonier et al. 2009). However, the baseline or stress-induced GC concentrations in plasma from laboratory and wild animals did not change in a consistent manner in response to chronic stress (Bonier et al. 2009; Dickens and Romero 2013) also reported inconsistent relationships between plasma GCs and fitness. Pawluski et al. (2017) suggested that the reported variability of GC concentrations under chronic stress may be due to the species studied, type of stressor or welfare measured, duration of stress, and techniques used to analyze GC concentrations.

Most moose in the present study had low to moderate deer ked intensities; only a few moose had high ked intensities. Paakkonen et al. (2012) found only minor effects of high infestation levels of deer ked on a range of systemic physiological measures, including plasma cortisol concentration (PCC). This contrasts with our findings. An explanation might be that the HCC measured in the current study is a more sensitive measurement of chronic stress than is PCC. In addition, HCC may remain less affected by variations in GC secretion in response to acute stress caused by hunting, which has the potential to mask small changes in GC caused by chronic stress compared to PCC.

TABLE 2. Hair cortisol concentration (HCC; pg/mg) as a function of deer ked (*Lipoptena cervi*) intensity (keds/cm^2) in Norwegian moose (*Alces alces*; *n*=262), using gamma distribution with inverse link. Because of the inverse link of the gamma distribution, a significant negative estimate refers to a positive association with HCC. The column ΔAICc refers to the change in the Akaike information criterion corrected for small sample sizes when the corresponding variable is excluded from the model. Deer ked intensity is ln transformed, centered around mean, and scaled to variance 1. The reference level (Intercept) is adult females at mean ln deer ked intensity (Intensity). The nonlinear relationship between deer ked intensity and HCC is modeled as a smooth spline (s), as shown in Figure 1A, and reported by the estimated degrees of freedom and the significance of the smooth term.

	Estimate	SE	*t* value	*P*	ΔAICc
Intercept	0.697	0.0442	15.8	<0.001	
Age calf vs. adult	−0.093	0.057	−1.63	0.105	12.8
Age yearling vs. adult	0.172	0.058	2.98	0.003	
Sex male vs. female	−0.178	0.048	−3.70	<0.001	13.3
s (ln Intensity)	2.90			<0.001	21.9

Hair may accumulate GC hormones over weeks to months, and although HCC is thought to be insensitive to the impact of acute stress, some evidence suggests that capture method may influence HCC (Cattet et al. 2014). Koren et al. (2019) found correlation between hair and serum cortisol in samples from moose. However, several studies have suggested that local cortisol production may contribute to GC integration in hair, because of a parallel, but peripheral, stress axis within the hair follicles (Ito et al. 2005; Keckeis et al. 2012). This does not imply that steroids measured in hair reflect local production only, but that this process may be independently influenced by both the central and peripheral stress axes (Cattet et al. 2014). Salaberger et al. (2016) found that mechanical irritation significantly increased HCC in sheep. Skin irritation caused by deer keds may have induced local GC production and metabolism in hair follicle cells regulated by locally expressed HPA mediators (Stubsjøen et al. 2015).

The lack of association between deer ked intensity and BM suggests that keds have minimal impact on moose foraging and metabolism early in the infestation period. However, we cannot rule out the possibility that deer keds may significantly impact moose during late winter when food is limited, the weather is harsh, and the parasites have exploited the host for a longer period of time.

Our results suggest that moderately intense deer ked infestations do not cause major chronic stress or loss of BM during the early infestation period of the deer ked. However, the increase in HCC at higher ked intensities even during this early parasitic phase of the infestation may be indicative of an increasingly negative response later in the infestation, affecting animal welfare negatively.

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TABLE 3. Hair cortisol concentration (HCC; pg/mg) as a function of deer ked (*Lipoptena cervi*) intensity (keds/cm^2) for the subset of moose (*Alces alces*; *n*=93) with deer ked intensity higher or equal to mean intensity. The column ΔAICc refers to the change in the Akaike information criterion corrected for small sample sizes when the corresponding variable is excluded from the model. Deer ked intensity is ln transformed, centered around mean, and scaled to variance 1.

	Estimate	SE	*t* value	*P*	ΔAICc
Intercept	0.682	0.035	19.5	<0.001	
ln Intensity	−0.097	0.029	−3.3	0.001	6.9

TABLE 4. Body mass (kg) in Norwegian moose (*Alces alces*; *n*=262), modelled as a spline of ln deer ked (*Lipoptena cervi*) intensity (keds/cm^2), using gamma distribution with a log link. When accounting for sex and age class, there was no significant association with body mass and deer ked intensity. Deer ked intensity is ln transformed, centered around mean, and scaled to variance 1. The reference level (Intercept) is female adults at population average of ln deer ked intensity. The smooth term (spline function [s]) of deer ked intensity (see Fig. 1B) is reported by the estimated degrees of freedom and the significance.

	Estimate	SE	*t* value	*P*
Intercept	5.145	0.032	161.6	<0.001
Age calf vs. adult	−0.902	0.052	−17.3	<0.001
Age yearling vs. adult	−0.343	0.039	−8.8	<0.001
Sex male vs. female	0.125	0.036	3.5	<0.001
s (ln Intensity)	1			0.15

excellent technical assistance in the laboratory.

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