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Assessment of chronic stress in sheep (part I): The use of cortisol and cortisone in hair as non-invasive biological markers

Solveig M. Stubsjøen[a,b,f,*], Jon Bohlin[a,c,g], Ellen Dahl[a,f], Maren Knappe-Poindecker[a,f], Terje Fjeldaas[a,f], Michael Lepschy[d], Rupert Palme[d], Jan Langbein[e], Erik Ropstad[a,f]

[a] Norwegian University of Life Sciences, P.O. Box 8146 Dep., NO-0033 Oslo, Norway
[b] Norwegian Veterinary Institute, Department of Health Surveillance, Section for Disease Prevention and Animal Welfare, P.O. Box 750 Sentrum, N-0106 Oslo, Norway
[c] Norwegian Institute of Public Health, Division of Epidemiology, Marcus Thranes Gate 6, P.O. Box 4404, 0403 Oslo, Norway
[d] University of Veterinary Medicine, Department of Biomedical Sciences/Unit of Physiology, Pathophysiology and Experimental Endocrinology, Veterinärplatz 1, 1210 Vienna, Austria
[e] Leibniz Institute for Farm Animal Biology, Institute of Behavioural Physiology, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany
[f] Department of Production Animal Clinical Sciences, P.O. Box 8146 Dep., NO-0033 Oslo, Norway
[g] Department of Food Safety and Infectious Biology, P.O. Box 8146 Dep., NO-0033 Oslo, Norway

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ABSTRACT

The ability to evaluate the extent of poor welfare during clinical disease is of great importance for animal welfare. Chronic exposure to stressful situations has a negative impact on animal welfare, and there is a need for valid and reliable biomarkers of chronic stress. The aim of this study was (1) to evaluate hair cortisol (HC) and hair cortisone (HCn) as measures of chronic stress in sheep and (2) to assess stress and pain associated with ovine footrot using different physiological [fecal cortisol metabolites (FCM), HC and HCn] and behavioral measures. The study was performed as a single foot inoculation using a boot. The right hind foot of 24 lambs was inoculated with different strains of *Dichelobacter nodosus*, and the animals were subsequently monitored daily for two weeks to assess lameness and pain. Hair was collected from both hind limbs at the start of the study and before treatment with gamithromycin subsequent to the trial period, and fecal samples were collected weekly for four weeks. Clinical signs of footrot developed in all experimental groups. There was an increase in FCM from week 0 to 2 ($p < 0.001$), and then a tendency of a subsequent decrease from week 2 to 3 ($p = 0.06$), indicating a chronic stress response due to the maceration caused by the boot and the developing infection. FCM decreased to baseline levels after the animals were treated with gamithromycin. Surprisingly, the concentration of HC decreased from week 0 to 3 ($p < 0.001$) in the right and left limb, and significantly more in the right limb ($p < 0.01$). The concentration of HCn increased from week 0 to 3 ($p = 0.05$) in the right limb but decreased in the left limb ($p < 0.05$). Hence, our findings suggest local production and/or metabolism of glucocorticoids in the hair follicles, which should be taken into consideration in studies using HC as a parameter of chronic stress. This study provides a first indication for a potential merit of hair cortisone as a biomarker of stress in sheep.

1. Introduction

Lameness has been a major concern to sheep farmers for centuries, and ovine footrot is one of the most common causes of lameness in sheep. *Dichelobacter nodosus* has been identified as the main causative agent (Roger, 2008). The first stage of the disease is an interdigital dermatitis that may progress to necrotic separation of the claw capsule from underlying tissues. The clinical symptoms of footrot include lameness, reluctance to move and reduced feed intake (Roger, 2008). Footrot has considerable consequences for animal welfare and cause economic losses for the sheep industry. Inflammatory diseases such as footrot are probably the major source of pain in ruminant species (Fitzpatrick et al., 2006). It is therefore vital to evaluate the extent of poor welfare during clinical disease (Broom and Corke, 2002). In this regard, non-invasive,

* Corresponding author at: Norwegian Veterinary Institute, P.O. Box 750 Sentrum, N-0106 Oslo, Norway. Fax: +47 23 21 63 01.
E-mail address: solveig-marie.stubsjoen@vetinst.no (S.M. Stubsjøen).

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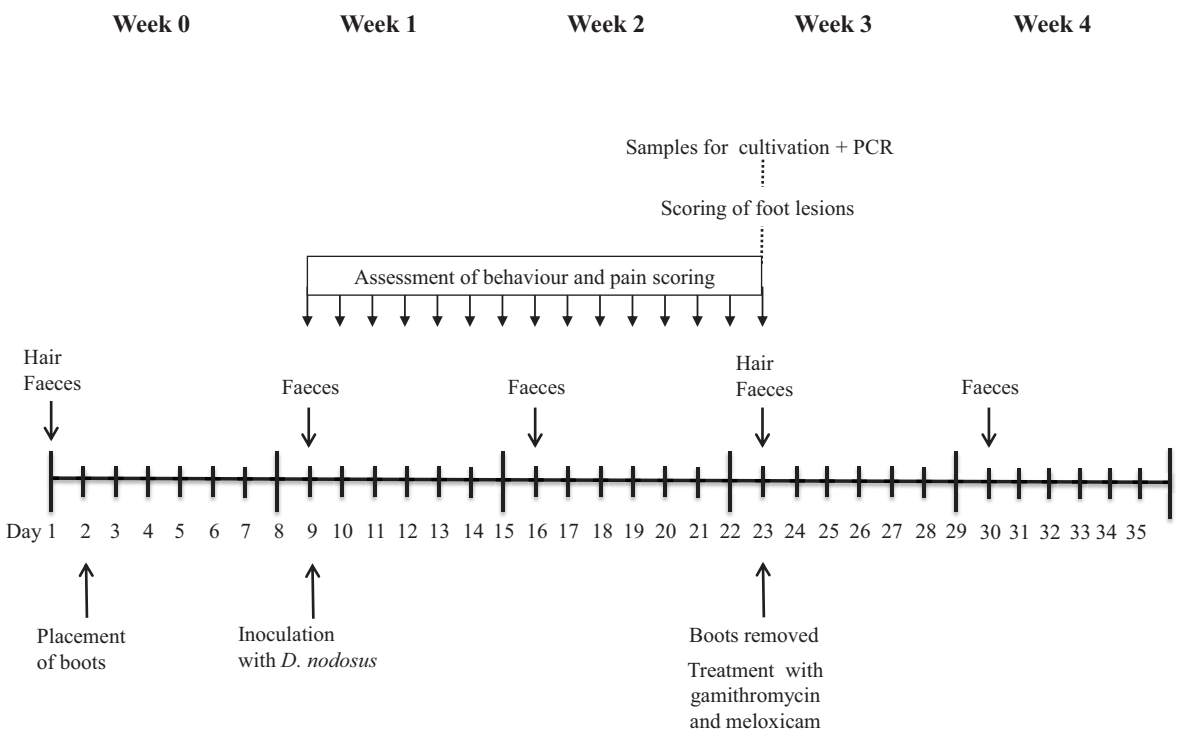

Fig. 1. The time line of the performed experiment. Baseline measures were conducted on Day 1 and 2 (Week 0). The lambs were inoculated with different strains of *D. nodosus* on day 9 (Week 1). The boots were removed on Day 23 (Week 3), and a range of samples and measures were collected before the lambs were treated for footrot. Fecal samples were also collected in Week 4 to assess the effects of treatment with gamithromycin.

objective methods to quantify stress and pain are indispensable tools to evaluate the welfare impact of diseases.

Cortisol has traditionally been measured in various body fluids and excreta in order to assess stress and pain in animals (Palme, 2012). Especially, the quantification of glucocorticoid metabolites in feces has been used as a non-invasive method to assess stress in a variety of species (Palme, 2012; Keckeis et al., 2012; Touma and Palme, 2005; Kleinsasser et al., 2010; Lepschy et al., 2010; Arias et al., 2013). The results remain unaffected by GC secretion in response to handling stress (Möstl and Palme, 2002) and the samples are easy to collect. This technique has previously been validated in sheep (Palme and Möstl, 1997; Palme et al., 1999; Möstl et al., 2002). However, measured cortisol (metabolites) in body fluids and excreta do not reflect the overall stress response over longer periods of time (Palme, 2012).

In recent years, the use of hair cortisol as a biomarker of chronic stress has attracted much attention (Accorsi et al., 2008; Gow et al., 2010; Russell et al., 2012; Ghassemi Nejad et al., 2014). Hair cortisol is insensitive to the impact of acute stress, including that caused by handling during sampling procedures. The procedure of collecting hair is simple, and the samples can be stored at room temperature and sent through the mail (Gow et al., 2010). Hair is a biomaterial that may accumulate glucocorticoid (GC) hormones over weeks to months (Gow et al., 2010). Three main models for compound incorporation into hair have been proposed: (1) active or passive diffusion from blood into growing cells in hair follicle, (2) diffusion from body secretion (sweat, sebum) during formation of hair shaft and (3) external environmental sources after hair shaft formation (Gow et al., 2010). Local cortisol production may participate as well. Indeed, Ito et al. (2005) demonstrated that hair follicles contain a functional equivalent of the hypothalamic–pituitary–adrenal (HPA) axis and can synthesize cortisol after stimulation by corticotrophin-releasing hormone. This finding has been supported by other studies (Sharpley et al., 2009; Keckeis et al., 2012) and may contribute to differences in hair cortisol concentrations found between different body locations (Moya et al., 2013). The mediators of this peripheral HPA axis are proposed to regulate the cutaneous response to local stressors (Slominski et al., 2007).

In addition, cortisol can be inactivated locally by an 11β-hydroxysteroid-dehydrogenase resulting in the formation of cortisone (Vanaelst et al., 2013). Although hair cortisone has received little attention so far, previous studies have indicated that cortisone may be a useful additional biomarker for stress research in hair (Raul et al., 2004; Perogamvros et al., 2010; Stalder et al., 2013; Vanaelst et al., 2013). Hence, further studies elucidating the incorporation of cortisol and cortisone into hair, and the usefulness of these glucocorticoids as parameters of chronic stress, are urgently needed.

The aim of this study was therefore: (1) to evaluate hair cortisol (HC) and cortisone (HCn) as measures of chronic stress in sheep and (2) to assess stress and pain associated with ovine footrot using different physiological [fecal cortisol metabolites (FCM), HC and HCn] and behavioral measures.

2. Material and methods

This study was conducted as part of a larger study which aimed to generate knowledge about ovine footrot in Norway by studying the effect of experimental infection with different strains of *D. nodosus* (Knappe-Poindecker et al., 2014). The protocol and conduct of this study was approved by the National Animal Research Authority in Norway (protocol number 11/3554).

2.1. Animals and housing

Twenty seven lambs of the breed Norwegian White, aged 4–5 months, with a mean body weight of 44 kg (range: 33–54 kg) were used in this study. Treatment groups of six lambs were used, and two cattle strains and two sheep strains (benign and virulent) of *D. nodosus* were tested. There were 3 males and 3 females in each treatment group, while the 3 lambs in the control group were males. The lambs were selected randomly from the experimental sheep flock free of footrot and *D. nodosus* at the Norwegian University of Life Sciences. The claws were clinically examined and samples were analyzed using PCR with regards to *D. nodosus* 8 weeks before the