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Leukocyte profile, gene expression, acute phase response, and metabolite status of cows with sole hemorrhages

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ABSTRACT

Sole hemorrhages result from disruption to normal claw horn formation and are caused by a variety of internal and external factors. Evidence suggests that they are painful, although they do not usually cause clinical lameness and are difficult to detect by observing cow gait. Little is known about how or whether sole hemorrhages affect the cow systemically. This study compared hematology profile, leukocyte gene expression, and physiological responses of cows with no/mild hemorrhages (category 1; n = 17), moderate hemorrhages (category 2; n = 18), and severe hemorrhages (category 3; n = 12). At approximately 100 d in milk, all cows in the study herd (n = 374) were locomotion scored before hoof examination. The cows included in the study were not clinically lame and had no other hoof disorder. Blood samples were taken from all cows within 24 h of selection. Leukocyte counts were obtained using an automated cell counter, cortisol and dehydroepiandrosterone (DHEA) concentration by ELISA, and plasma haptoglobin, urea, total protein, creatine kinase and glucose were analyzed on a clinical chemistry analyzer. Expression of 16 genes associated with lameness or stress were estimated using real-time quantitative PCR. Data from cows within each category were compared using the Mixed procedure in SAS (version 9.3; SAS Institute Inc., Cary, NC). Fixed effects included hemorrhage severity category and lactation number, with days in milk and body condition score included as covariates. Locomotion score worsened as sole hemorrhage category worsened. Locomotion score of category 1 cows tended to be lower than that of category 2 cows and was lower than that of category 3 cows. The locomotion score of category 3 cows was also greater than that of categories 1 and 2 combined. Category had no effect on leukocyte number, on any of the individual leukocyte cell numbers or percentages, cortisol or DHEA concentration, cortisol:DHEA ratio, or relative expression of any of the genes investigated, and we detected no differences in plasma glucose, protein, or creatine kinase concentrations between categories. However, category 3 cows had greater plasma concentrations of haptoglobin and tended to have lesser concentrations of plasma urea than category 1 and 2 cows. The differences in gait between cows with no or minor sole hemorrhages and cows with severe hemorrhages indicate that hemorrhages may be associated with discomfort or pain. Nevertheless, the only physiological measure that changed with increasing locomotion score was plasma haptoglobin concentration. Haptoglobin has previously been found to be elevated in lame cows, and thus shows promise as a marker for limb pain.
Key words: sole hemorrhage, immune, haptoglobin, locomotion

INTRODUCTION

Disruption to the formation of dairy cow hoof horn causes tissue damage that can result in a variety of claw horn disruption lesions. These include sole hemorrhages, which vary in severity from very faint yellow or pink patches of horn to extremely dark red areas. Sole hemorrhages are the visible evidence of disruption of blood flow to the corium, the part of the hoof responsible for synthesizing claw horn (Bicalho and Oikonomou, 2013). This disruption is thought to be caused by excessive movement of the third phalanx inside the hoof capsule, which in turn results from exposure to a variety of risk factors, such as hormonal changes around parturition, a thin digital cushion, long walking distances, or management in facilities with poor cow comfort (Bicalho and Oikonomou, 2013). Severe disruption to the corium can result in hoof horn damage to the extent that underlying tissue is exposed at the surface of the hoof, which is known as a sole ulcer. Thus, hemorrhages may be considered an early stage or minor version of sole ulcers (Bergsten, 2004).

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Sole ulcers are one of the most severe hoof pathologies and are normally associated with clinical lameness (O'Driscoll et al., 2015). Clinical lameness has been demonstrated to be associated with a sickness response (Whay et al., 1997; Almeida et al., 2008), involving changes to both behavior and physiology and regulated by cytokines released by immune cells (Dantzer and Kelley, 2007). More specifically, O'Driscoll et al. (2015) found indications of systemic inflammation in cows that were lame only due to the presence of sole ulcers. For instance, cows with sole ulcers had a greater neutrophil: lymphocyte ratio, and greater concentrations of serum cortisol and dehydroepiandrosterone (**DHEA**) than cows without sole ulcers. Relative expression of several cytokine genes were more highly expressed in cows with ulcers than those without (*IL-1a*, *IL-1β*, *CXCL8*, and *IL-10*), as was relative expression of *MMP-13*, *GR-a*, *FAS*, *HP*, and *CD62L*. Increased expression of *MMP-13* mRNA has been found in cows with impaired locomotion (Almeida et al., 2007) and the gene could be implicated in development of claw horn disorders.

Although sole hemorrhages represent damage to the claw, the damage is not as severe as that caused by sole ulcers. Moreover, sole hemorrhages are not always associated with clinical lameness or indeed even easily detectable impairment to locomotory ability (Flower and Weary, 2006). Nevertheless, the underlying etiology for hemorrhages and ulcers is similar, and it is possible that hemorrhages are also associated with pain and immune system activation. Although it can be difficult to identify cows with sole hemorrhages by observing their gait (Flower and Weary, 2006), there is evidence of subclinical hoof pain in cows, which does not manifest in altered locomotion (Dyer et al., 2007). In the Dyer et al. (2007) study, the threshold of claw pain decreased nearly 30% below normal before there were any visible changes to locomotory ability. Pastell et al. (2010) reported that measurement of the ratio of weight applied to a pair of legs was reasonably able to discriminate between cows with or without sole hemorrhages, even though it was less successful at discriminating between cows with or without ulcers. Pastell et al. (2010) suggested that the level of pain associated with sole hemorrhages might vary depending upon the location on the foot or whether or not they were sole ulcers in the initial stage of development.

The objective of this study was to characterize the effects of sole hemorrhage severity on leukocyte profile, cortisol and DHEA response, metabolite profile, and expression of genes associated with lameness and stress. We hypothesized that increases in sole hemorrhage severity would be associated with increased expression of measures associated with lameness and activation of the immune system, even when the hemorrhages did not result in impaired gait. The results will contribute to knowledge regarding systemic effects of solar hemorrhaging and the severity of the disorder.

MATERIALS AND METHODS

All animal procedures performed in this study were conducted under experimental license (B100/4319) from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005. The study animals were located at the Teagasc Moorepark research farm, part of the Animal and Grassland Research and Innovation Centre (Teagasc, Moorepark, Fermoy, Co. Cork, Ireland). Samples were collected between April 2011 and February 2012. Laboratory analyses were carried out at the Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre (Teagasc, Grange, Dunsany, Co. Meath, Ireland).

Animals and Enrollment in the Study

All cows from the Moorepark herd (~375 cows) were observed individually walking from the milking parlor after morning milking at approximately 100 d postpartum [104 ± 23 (mean ± SD) DIM; range 76 to 162]. They then had their hooves lifted and examined. Locomotion and hoof scoring was carried out by a single trained observer throughout the study. Only cows that were not clinically lame (defined as any obvious impairment to gait, including the cow being unwilling or slow to place one or more feet on the ground) but with sole hemorrhages of varying severity were used in the study. Individual cow records were examined using the herd recording system to ensure that the selected cows had remained free from health disorders during their current lactation. The final cohort included in the analysis consisted of 47 primiparous (n = 14) and multiparous (n = 33; parity range 2 to 7; mean 3.5 ± 1.3) Holstein-Friesian cows. Average milk yield, liveweight, and BCS [ranging from 1 (emaciated) to 5 (extremely fat) with increments of 0.25; Edmonson et al., 1989] at the time of taking experimental measures were 23.2 ± 4.5 kg/d per cow, 509 ± 65 kg, and 2.78 ± 0.23, respectively.

None of the enrolled cows were assigned to experimental treatments at the time of this study. The majority of the cows (n = 41) were enrolled while being kept on pasture during the summer (April to August 2011). These cows were fed a grass-based diet (approximately 80% perennial ryegrass) and managed using the