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## Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



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### Coding and quantification of a facial expression for pain in lambs

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#### ARTICLE INFO

SEVIER

Article history: Received 20 January 2016 Received in revised form 5 July 2016 Accepted 27 September 2016 Available online 28 September 2016

*Keywords:* Facial expression Grimace Lamb Pain

#### ABSTRACT

Facial expressions are routinely used to assess pain in humans, particularly those who are non-verbal. Recently, there has been an interest in developing coding systems for facial grimacing in non-human animals, such as rodents, rabbits, horses and sheep. The aims of this preliminary study were to: 1. Qualitatively identify facial feature changes in lambs experiencing pain as a result of tail-docking and compile these changes to create a Lamb Grimace Scale (LGS); 2. Determine whether human observers can use the LGS to differentiate tail-docked lambs from control lambs and differentiate lambs before and after docking; 3. Determine whether changes in facial action units of the LGS can be objectively quantified in lambs before and after docking; 4. Evaluate effects of restraint of lambs on observers' perceptions of pain using the LGS and on quantitative measures of facial action units. By comparing images of lambs before (no pain) and after (pain) tail-docking, the LGS was devised in consultation with scientists experienced in assessing facial expression in other species. The LGS consists of five facial action units: Orbital Tightening, Mouth Features, Nose Features, Cheek Flattening and Ear Posture. The aims of the study were addressed in two experiments. In Experiment I, still images of the faces of restrained lambs were taken from video footage before and after tail-docking (n=4) or sham tail-docking (n=3). These images were scored by a group of five naïve human observers using the LGS. Because lambs were restrained for the duration of the experiment, Ear Posture was not scored. The scores for the images were averaged to provide one value per feature per period and then scores for the four LGS action units were averaged to give one LGS score per lamb per period. In Experiment II, still images of the faces nine lambs were taken before and after tail-docking. Stills were taken when lambs were restrained and unrestrained in each period. A different group of five human observers scored the images from Experiment II. Changes in facial action units were also quantified objectively by a researcher using image measurement software. In both experiments LGS scores were analyzed using a linear MIXED model to evaluate the effects of tail docking on observers' perception of facial expression changes. Kendall's Index of Concordance was used to measure reliability among observers. In Experiment I, human observers were able to use the LGS to differentiate docked lambs from control lambs. LGS scores significantly increased from before to after treatment in docked lambs but not control lambs. In Experiment II there was a significant increase in LGS scores after docking. This was coupled with changes in other validated indicators of pain after docking in the form of pain-related behaviour. Only two components, Mouth Features and Orbital Tightening, showed significant quantitative changes after docking. The direction of these changes agree with the description of these facial action units in the LGS. Restraint affected people's perceptions of pain as well as quantitative measures of LGS components. Freely moving lambs were scored lower using the LGS over both periods and had a significantly smaller eve aperture and smaller nose and ear angles than when they were held. Agreement among observers for LGS scores were fair overall (Experiment I: W=0.60; Experiment II: W = 0.66). This preliminary study demonstrates changes in lamb facial expression associated with pain. The results of these experiments should be interpreted with caution due to low lamb numbers.

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http://dx.doi.org/10.1016/j.beproc.2016.09.010 0376-6357/© 2016 Elsevier B.V. All rights reserved.

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#### 1. Introduction

Most mammals can change their facial expression in response to a range of stimuli or experiences (Diogo et al., 2009). Mammalian facial expressions may serve an adaptive function, whereby information about emotion, intent or the environment can be sent to a nearby observer. In humans, describing facial expression is nearly synonymous with describing emotion (Waller and Micheletta, 2013). Several studies identify a specific facial expression, or grimace, for pain in humans. This can be described in terms of brow lowering, cheek raise, eyelid tightening, nose wrinkle and eye closing (Craig, Prkachin, and Grunau 1992; Prkachin, 1992). Identifying human pain via facial grimace is useful, as it allows clinicians to assess pain in non-verbal patients (Hicks et al., 2001).

Recently, there has been interest in developing coding systems for grimacing in non-human mammals. A Mouse Grimace Scale (MGS) was developed using the same method used to identify pain related changes in human facial expressions. Photographs of mice from before and after a range of routine nociceptive tests (for example administration of an irritating substance into the abdominal cavity) were compared to identify which facial features changed in response to these painful procedures (Langford et al., 2010). The MGS consists of five facial action units: orbital tightening, nose bulge, cheek bulge, ear position and whisker change. Rat and Rabbit Grimace Scales were subsequently developed in a similar manner (Sotocinal et al., 2011; Keating et al., 2012), with the majority of action units being broadly similar to those of the MGS with the exception of cheek flattening in rats and rabbits as opposed to bulging in mice. The Horse Grimace Scale has been developed using animals undergoing routine castration. This study identified six facial action units: stiffly backward ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin, and strained nostrils and flattening of the profile (Dalla Costa et al., 2014). Similar changes were noted in horses when a tourniquet was applied on the antebrachium or with the application of an irritant, but were described as low ears, angled eye, withdrawn and tense stare, square-like nostrils, tension of the muzzle, and tension of the mimic muscles (Gleerup et al., 2015).

Facial expressions of pain in these species are consistently recognized by human observers as all scales demonstrated high inter-observer reliability and accuracy. Further studies have been conducted to validate these scales by using them to assess post-procedural pain and efficacy of routinely used analgesics as well as by comparing the findings to behavioural and physiological indicators of pain (Keating et al., 2012; Leach et al., 2012; Dalla Costa et al., 2014; Gleerup et al., 2015; Matsumiya et al., 2012).

Recently, a facial expression scale was developed for sheep with naturally-occurring pain due to foot-rot. Consistent with scales for other mammals, the Sheep Pain Facial Expression Scale describes changes in orbital tightness, cheek tightness, ear position, lip and jaw profile, and nostril and philtrum position (McLennan et al., 2016). Lambs may also show a noticeable change in facial expression due to acute pain. Domestic lambs experience pain routinely as they undergo painful husbandry procedures including ear tagging, tail docking and castration (Mellor and Stafford, 2000).

The aims of this preliminary study were to: 1. Qualitatively identify facial feature changes in lambs experiencing pain as a result of tail-docking and compile these changes to create a Lamb Grimace Scale (LGS); 2. Determine whether human observers can use the LGS to differentiate tail-docked lambs from control lambs and differentiate lambs before and after docking; 3. Determine whether changes in facial action units of the LGS can be objectively quantified in lambs before and after docking; 4. Evaluate effects of restraint of lambs on observers' perceptions of pain using the LGS and on quantitative measures of facial action units.

#### 2. Methods

The Massey University Animal Ethics Committee approved all procedures for both experiments (Protocol 12/104). Tail-docking occurred as part of routine husbandry practices in New Zealand and in accordance with the codes of practice outlined in the Painful Husbandry Procedures Code of Practice (Anon, 2005). Both experiments were undertaken at the Massey University Keebles Farm in Palmerston North, New Zealand.

#### 2.1. Experiment I

#### 2.1.1. Animals

Nine 5 to 6 week old Romney cross lambs were used in this study (four females and five males). Lambs were randomly selected from a flock of 40 lambs and their dams. Prior to testing, the ewes and lambs were kept on pasture according to normal husbandry practice.

#### 2.1.2. Experimental procedure

Testing was undertaken in an outdoor yard with concrete floors. On the day of testing, the dams and lambs were brought in from the paddock as a flock and kept in a holding yard. One lamb at a time was randomly selected for testing. The same experimenter picked the lambs up and held them in a seated position for the duration of tail docking and subsequent observation. All lambs were tested over one day.

Lambs were alternately allocated to one of two treatments: they were either tail-docked using a rubber ring or sham-docked (control). The treatments were applied by the farm manager and the lambs were restrained for the duration of observation. The rubber ring was applied using an elastrator between two tail vertebrae at a point allowing sufficient tail proximal to the ring to cover the anus (and vulva for female lambs). During sham docking the farm manager handled the tail area for 15 s, to replicate docking without the ring being applied. The lamb's face was recorded for 1 min before (pre) and 10 min after (post) treatment using a high definition video camera from the front angle (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd., Tokyo, Japan). Ten minutes after the treatment was applied, the lambs were released back to the paddock.

Two lambs were excluded from the analysis. One received an ear notch prior to filming, which may have altered its response to tail docking. The second had been allocated to the control (sham-docking) treatment group and fell asleep during the handling procedure and hence its facial expression may have been incorrectly interpreted (Langford et al., 2010; Sotocinal et al., 2011). Therefore, data from seven lambs were analyzed (3 control (1 female), 4 docked (2 female)).

#### 2.1.3. Frame capture

For each lamb, four still images were extracted manually from the video recordings for each period (pre- and post-treatment) to produce 8 images per lamb. Stills were selected from across the 1 min pre-docking period and in the last 5 min of the posttreatment period, as freely behaving lambs show a high frequency of other pain-related behaviours around this time after docking (Molony and Kent, 1997). Images were selected every 15 s across the 1 min pre-period and every 75 s in the post-period. When a lamb was moving at that particular moment the image was taken immediately after or before the selection time point. We attempted to blind the person selecting frames to the treatment group by randomly numbering the videos used for selection, however due to Download English Version:

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