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Highlights

SPFES can accurately identify sheep with painful diseases from healthy sheep.

Trained observers reliably and accurately used the SPFES to detect pain in sheep.

Treatment of disease reduced the total facial pain score of adult sheep.

Total pain scores positively correlated with lesion and lameness scores.

Development of a facial expression scale using footrot and mastitis as models of pain in sheep.

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Abstract

Management of pain in sheep is limited by the challenges of recognising and accurately quantifying pain in this species. The use of facial expression scoring to assess pain is a well-utilised, practical tool in both humans and non-human animals. The objective of this study was to develop a standardised facial expression pain scale for adult sheep, that could be used reliably and accurately to detect pain associated with naturally occurring painful diseases, such as footrot and mastitis. We also investigated whether the scale could be reliably and accurately utilised by observers after training, enabling the development of an on-farm pain assessment tool. The Sheep Pain Facial Expression Scale (SPFES) was able to correctly identify sheep suffering from disease with a high degree of accuracy (AUC; Footrot: 0.81, Mastitis: 0.80). Diseased sheep scored higher on the scale than controls on the day of disease identification ($P<0.05$) and diseased sheep showed changes in their facial expression after treatment ($P<0.001$). The abnormal facial expressions of diseased sheep reduced over time, and at recovery were in line with control sheep. Control sheep did not change their facial expression over time. Five scorers who were trained to use the developed scale also assessed the facial expressions of sheep. The scorers were blind to treatment and session. Scorers reliably (ICC: 0.86) and accurately ($\alpha = 0.86$) identified changes in the facial expression of sheep with footrot over time ($P<0.05$), and scored control sheep consistently low over time. The SPFES offers a reliable and effective method of assessing pain in sheep after minimal training.

Keywords:

Footrot; Sheep; Pain; Facial expression.

1. Introduction

Pain is an aversive experience with both sensory and affective components, often associated with actual or potential tissue damage (Broom, 2001; IASP, 1994; Sneddon et al., 2014). Pain can have considerable effects on the well-being of an animal and its quality of life. The management of pain in farm animals however, is often inadequate, resulting in poor welfare (Crook, 2014; Huxley and Whay, 2006). Reasons commonly cited by veterinarians for not administering analgesia to farm animals include cost to the farmer, withdrawal periods for drug residues and a lack of licensed analgesic products in some animals such as sheep (although they can be used under “The Cascade System”) (Lizarraga and Chambers, 2012). One of the major reasons limiting the use of pain relieving drugs in farm animals is difficulties in recognising and quantifying pain (Flecknell, 2008; Huxley and Whay, 2006; Ison and Rutherford, 2014; Lizarraga and Chambers, 2012). There is an evident need for an objective, reliable scoring tool that can be effectively used to recognise and assess pain severity in sheep.

Disease is a major source of pain in sheep, impacting negatively upon welfare and adversely effecting productivity. Footrot in sheep causes severe lameness, a direct sensory response to the tissue damage caused by the bacteria *Dichelobacter nodosus* (Kaler et al., 2010a). As lesion severity increases the degree of lameness observed also increases, indicating the presence of pain (Dolan et al., 2003; Kaler et al., 2010b). Mechanical threshold responses are also significantly reduced when severe footrot is present, indicating the presence of chronic pain; the application of a local anaesthetic block raises the threshold to be in line with that of healthy sheep (Ley et al., 1989). Resolution of the lesions does not necessarily remove this pain, as hyperalgesia to a mechanical stimulus may still be present for up to three months in sheep that had previously suffered from severe footrot (Dolan et al., 2003; Ley et al., 1989).

Mastitis is also regarded as an extremely painful disease in sheep. Mastitis is the inflammation of the mammary glands usually in response to pathogens such as *Staphylococcus aureus* and *Mannheimia haemolytica* (Jones, 1991). These pathogens can also cause painful lesions within the teat canal (Mavrogianni et al., 2004). The development of the disease can be rapid, and in severe cases can lead to death of the sheep. Sheep with mastitis also show mechanical hyperalgesia (Dolan et al., 2000), which supports the hypothesis that this is a painful condition.

Non-steroidal anti-inflammatory drugs (NSAIDs) have antipyretic, anti-inflammatory and analgesics properties. This supports their use alongside antimicrobials in treating inflammatory conditions such as footrot and mastitis, to aid recovery and reduce the associated pain. Within veterinary practice, sheep suffering from mastitis are more likely to receive an NSAID as part of their treatment as it can rapidly reduce clinical signs of mastitis (Fthenakis, 2000). There is some evidence to also support the use of NSAIDs when treating sheep with footrot; Welsh and Nolan (1995) administered an NSAID, flunixin meglumine, to sheep suffering from footrot. They found mechanical hyperalgesia to be reduced in these sheep compared with sheep that did not receive an NSAID, demonstrating its analgesic property. Kaler et al. (2010a) assessed the anti-inflammatory property of flunixin meglumine as an aid to recovery in sheep with footrot. However, they did not find any effect of NSAIDs on time to recovery when compared with sheep that only received an antibiotic. In sheep, meloxicam has a longer elimination half-life than flunixin meglumine (10.85 ± 1.21 h, 2.48 ± 0.12 h respectively) (Cheng et al., 1998; Shukla et al., 2007) and is detectable in blood plasma for up to 72 hours (Shukla et al., 2007) compared to 32 hours for flunixin meglumine (Cheng et al., 1998). These studies provide evidence for meloxicam to be a better alternative to flunixin meglumine in reducing inflammation and pain associated with diseases such as footrot and mastitis in sheep. The effect of meloxicam as an NSAID has not yet been assessed for its ability to reduce pain associated with disease in sheep.

Current pain assessment tools commonly use behavioural indicators as these provide sensitive and non-invasive measures of pain (Mogil and Crager, 2004). Pain related behaviours such as lip curling, trembling, abnormal postures and vocalisations have been well documented when assessing pain in lambs undergoing tail docking and castration (Grant, 2004; Guesgen et al., 2014; Molony et al., 2002). Observing behavioural changes can be time consuming, making it impractical for on-farm settings. Furthermore, the fluctuating nature of spontaneous pain can mean that smaller, more subtle changes are likely to be missed (Foss et al., 2006).

Facial expression scoring systems for pain assessment have been recently developed for use in rodents, rabbits and horses (Dalla Costa et al., 2014; Langford et al., 2010; Leach et al., 2012). Facial expression scoring has shown to be successful in identifying and assessing the severity of pain in animals, with minimal time and training required for observers (Langford et al., 2010; Leach et al., 2012; Sotocinal et al., 2011). Changes in facial expression are likely to be an involuntarily response by an animal in response to the fluctuating level of pain experienced (Langford et al., 2010) leading to higher sensitivity in the assessment. The evolutionary stability of facial expression across species (Williams, 2002) and their use within social contexts (Defensor et al., 2012), suggest that adult sheep would also be likely to exhibit changes within their facial expression when experiencing pain.

The objective of the present study therefore, was to develop a standardised facial expression pain scale that can be used accurately to detect pain associated with naturally occurring painful diseases such as footrot and mastitis. This objective was achieved by visiting eleven commercial farms across East Anglia, UK when disease was reported, and evaluating the changes in facial expressions before and after treatment with antibiotics and during the recovery time. Some of the sheep with footrot were also treated with an NSAID to evaluate the effect of initial analgesia on the expression of pain in sheep during recovery from the disease. We also tested whether the SPFES we developed could be reliably and accurately utilised by observers after training, and thus be a useful and practical on-farm pain assessment tool.

2. Methods

2.1 Ethical statement

Ethical approval was provided by the Department of Veterinary Medicine, University of Cambridge Ethics and Welfare Committee. All disease incidents were naturally occurring and all animals were under the supervision of a veterinarian. All sheep suffering from disease were treated appropriately and revisited throughout the recovery period. No treatment was withheld during the study. Stress to sheep was minimised when handling or approaching animals. Information was provided to each farmer before they gave consent for the study to commence on their farm. Informed consent was obtained from each observer prior to scoring images. All data was anonymised before analysis and no personal details of the participants were recorded or stored.

2.2 Footrot

2.2.1 Study population

One hundred and eleven sheep of differing breeds, gender and coat colour were involved in the study. All the sheep were over one year of age. A total of 73 sheep were diagnosed as having footrot by a veterinarian, using lameness and lesion scoring. These sheep were matched with 38 control sheep from the same farm that had no signs of footrot or other disease. Data were collected from October 2012 through to July 2014 across all seasons from eight farms.

2.2.2 Study design and treatments

All sheep were assessed for lameness using the five point gait scoring method devised by Ley et al. (1992). All sheep were assessed for footrot lesions using the four point scale developed by Egerton and Roberts (1971). Sheep were categorised into three treatment groups. Group FA (N=37) were treated for the presence of footrot with antibiotics, tulathromycin by subcutaneous injection (2.5mg/kg Draxxin®, Zoetis, Ltd) and topical chlortetracycline (Animedazon® Spray 2.4%, AniMedica). Group

FAN (N=36) were treated for the presence of footrot as before and also received a non-steroidal anti-inflammatory drug, meloxicam by sub-cutaneous injection (0.5mg/kg, Metacam®, Boehringer Ingelheim Ltd). Group FC (N=38) showed no signs of lameness and were clinically assessed as being free from clinical disease by a veterinarian and were used as controls. Controls were matched carefully on each farm for breed, gender and age.

Photographic images of sheep faces were taken on the day of disease identification (day 0) after lameness and lesions were scored. All sheep received an initial treatment on the same day (day 0) after images had been collected. All sheep were revisited during their recovery period and received additional treatment as required by the veterinarian, if signs of active disease were still present. Animals were reassessed for lesions and lameness to establish that they were fully recovered and facial images were recorded again on day 90.

2.3 Mastitis

2.3.1 Study population

Twenty nine primiparous and multiparous recently parturient ewes of differing breeds, coat colour and number of lambs were involved in the study. A total of 17 sheep were identified as having acute clinical mastitis by a veterinarian. These sheep were matched as closely as possible for days since parturition and for number of offspring, with a total of 12 control sheep from the same farm identified as having no signs of clinical mastitis. Data were collected over two lambing seasons (January to July) in 2013 and 2014 from four farms.

2.3.2 Study design and treatments

All sheep were assessed for signs of acute clinical mastitis through udder colour and udder palpation by a veterinarian. A milk sample from diseased sheep was taken to identify the pathogen and ensure correct treatment was applied. Sheep were categorised into two treatment groups. Group MAN (N=17) were treated with an appropriate antibiotic, either tulathromycin by subcutaneous injection (2.5mg/kg Draxxin®, Zoetis Ltd) or Oxytetracycline by intramuscular injection (10mg/kg Alamycin LA®, Norbrook Laboratories, Ltd), and all animals received a non-steroidal anti-inflammatory drug, meloxicam by subcutaneous injection (0.5mg/kg, Metacam®, Boehringer Ingelheim Ltd). Group MC (N=12) were assessed as being free from clinical disease by the veterinarian and were used as controls.

Photographic images of sheep faces were taken on the day of disease identification (day 0) after udders were assessed. All sheep were treated on the same day (day 0) after images had been recorded. All sheep were revisited during their recovery period and further images were collected on day 7 and again on day 42. Animals were reassessed for signs of clinical mastitis to ensure full recovery had occurred by day 42. If sheep had not responded to the initial treatment, further treatment was provided by the veterinarian. Sheep were assessed in small groups with their lambs and stress was kept to a minimum.

2.4 Image capture

Multiple photographs of sheep were taken from a distance of approximately 1 m using a high definition camera (Casio®, Exilim HS EX-ZR100, Casio Electronics Co., Ltd., Japan). Photographs were taken on day 0 after animals had been assessed for presence or absence of disease and had been left for twenty minutes to settle. Further photographs were taken on day 7 and 42 for mastitis and on day 90 for footrot before sheep were handled or reassessed for disease presence or absence.

Profile and frontal pictures were taken for each animal on each occasion whilst they remained within the group. All photographs were cropped to include only the head and to remove body posture, to prevent observers being influenced by the posture of the animal when scoring the facial expressions as in Langford et al. (2010) and Leach et al. (2012). The highest quality pictures were used for scoring where possible.

2.5 Sheep pain facial scale development

The sheep pain facial expression scale (SPFES) was developed using sheep from the footrot study group. Footrot was used as our pain model following previous research showing the link between lameness due to footrot and mechanical hyperalgesia (Ley et al., 1989). We followed methods by Langford et al. (2010), Sotocinal et al. (2011), Leach et al. (2012), Keating et al. (2012) and Dalla Costa et al. (2014) to develop our scale. Images of sheep on days 0 and days 90 were compared to identify changes in facial expression associated with the presence of the disease and lameness. Based on these comparisons an initial scale was established and trialled in a pilot study (McLennan et al., 2014). Minor adjustment to the scale with the addition of higher quality photographs and more detailed descriptors allowed the development of the SPFES (Fig. 1). The scale is used to assess expression within five facial areas; orbital tightness, cheek tightness, ear position, lip and jaw profile, and nostril and philtrum position. These areas are scored as having abnormal expression not present, partially present, or present.

2.6 Scoring facial expression.

The facial expressions of sheep from both footrot and mastitis were scored separately by an observer (KM) who was experienced in the use of the scoring system. To reduce possible bias, scoring took place three months after the scale had been finalised. Photographs that were not in focus or were of poor quality for angle and light were not scored. To maintain a balanced design, only sheep that had a complete set of photographs across all time points were included. Sheep that required more than one treatment were removed from further analysis. A total of 51 sheep from the footrot group (n, FA=16, FAN=19, FC=16) and 22 for mastitis (n, MAN =12, MC=10) were scored. The scores from KM were used to test the sensitivity and specificity of the scale at detecting disease status and thus pain for mastitis and footrot. The scores were also analysed to determine the effect of time, treatment and a time*treatment interaction.

Five treatment and session blind observers who had been given training on how to score the facial expressions of sheep, scored a sample data set of 60 images from the footrot group consisting of 20 sheep with footrot (n, FOA=9, FOAN=11) and 10 control sheep (FOC). Training consisted of viewing a pictorial guide with descriptors as well as multiple example images of each of the five facial areas. This file also included training and testing sections as well as instructions on how to fill out the scoring file. Training images were not used within the scoring file. The scores from these individuals after training were used to test the reliability and accuracy of the scale across each treatment group for the footrot population. The training tool can be found at www.animalwelfarehub.com.

Two photographs, one profile and one frontal, were assessed for each time point. Images were presented in a random order generated using a random number generation on Microsoft Excel 7.0. Both photographs were used to give one score to each of the facial areas using the three-point scale (0 = not present, 1 = partially present, 2 = present). If the two photographs differed in value, or one area was obscured from view (e.g. nostril and philtrum position can only be seen from the frontal view) the highest score of the two photographs was given. If an area was not clear on either of the photographs, it was scored as ‘not able to score’. If two or more areas were scored as ‘not able to score’, the total score for this image was not included in the analysis. A total pain score (TPS) was determined by adding the individual scores for each of the five areas for each set of photographs. The maximum possible score was 10 (i.e. a score of 2 for each of the 5 facial areas). The five observers were also asked to make a global assessment of whether they thought the sheep was in pain or not, based on their own previous experience, as used by Dalla Costa et al. (2014) and Keating et al. (2012).

2.7 Statistical analysis

Statistical analysis was carried out using Ri386 3.1.1 (R Core Team, 2014) except for receiver operator curve (ROC) analysis which was carried out using SPSS 22.0 (IBM Corp, 2013). Differences were considered statistically significant at $P \leq 0.05$ and results are reported as mean ± SEM unless where otherwise stated. Spearman's rank correlations were calculated to investigate the relationships between TPS, lameness and the total lesion scores, as this data was not normally distributed. Spearman's rank correlations were also calculated between each of the facial areas and the TPS. The sensitivity (ability of a test to correctly identify animals with the disease) and specificity (ability of a test to correctly identify animals without the disease) of the scale were calculated. Sensitivity is the ratio of true positives (*TP*) to true positives plus false negatives (*FN*): sensitivity = *TP/ TP+FN*. Specificity is the ratio of true negatives (*TN*) to true negatives plus false positives (*FP*): specificity = *TN/TN+FP*. ROC analysis was carried out by plotting for all cut-off points, the rate of false positives against the rate of true positives. A value of 1.0 indicates a perfect test, whilst a value of 0.5 indicates an inadequate test (Lalkhen and McCluskey, 2008). The sensitivity and specificity for each of the TPS levels was also determined. For footrot groups the outcome was lameness with the predictor as TPS. For the mastitis group sensitivity and specificity was calculated for the first day only with the outcome being disease status and the predictor as TPS. A repeated measures linear mixed-effects model fit by maximum likelihood was used to analyse the TPS across time points (footrot: day 0 and 90; mastitis: day 0, 7 and 42). Day, as the repeated measure was nested within sheep as the random effect, with treatment group, day, breed and farm as fixed effects. Any time*treatment interactions were further investigated using analysis of variance with data from separate time periods forming the dependent variables and treatment as the fixed effect. Post-hoc analysis of treatment group effects was conducted using Tukey contrast tests. The Kruskal-Wallis test was used to investigate time*treatment interactions for the footrot group on day 90 due to data being not normally distributed. Kruskal-Wallis was also used to investigate time*treatment interactions for the mastitis group for days 7 and 42 due to data not being normally distributed. In addition, changes in facial expression across days for each treatment group was calculated and compared to zero using a 1-sample t-test, or a Wilcoxon signed rank test where data were not normally distributed.

The global accuracy of the facial pain score was determined by comparing the global pain or no pain judgement made by treatment and session blinded scorers with the actual disease state of the sheep in each photograph based upon the lameness and lesion scores (e.g. control or diseased on day 0 and day 90). Reliability of the scale was assessed by comparing the participants' scores for each area and the TPS, using the intra-class correlation coefficient (ICC), Cronbach's alpha.

3. Results

3.1 Footrot

The TPS scores over the two time periods showed a good accuracy with the area under curve (AUC) reaching 0.81, compared to lameness. Table 1 shows the sensitivity and specificity of each total facial expression score. Table 2 gives details on the correlation between facial areas and the TPS. There were no significant main effects of sheep gender (P=0.47), breed (P=0.12) or farm (P=0.75) on TPS. Time, treatment and time*treatment had significant effects on TPS (P=0.0001, P=0.0007, P=0.0436 respectively). On day 0 TPS were significantly different between the three treatment groups ($F_{(2,48)}$ = 9.02, P=0.0005), with the TPS being higher in sheep with footrot (groups FA and FAN) compared to the control group (group FC) (Tukey post-hoc, P<0.01 for both comparisons). No differences were found between groups that received just antibiotics (FA) and those that received an additional non-steroidal anti-inflammatory drug (FAN) (Tukey post-hoc, P=0.86). At day 90 there were no significant differences between treatment groups (χ^2 = 4.59, df=2, P=0.10) (Fig. 2). Sheep that were treated for footrot with antibiotic only, had a decrease in their facial expression score from day 0 to day 90 (t=-3.29, df=15, P=0.005), as did sheep that received an additional non-steroidal anti-inflammatory drug (V=7.5, P=0.003). Control sheep did not have a change in their facial expression from day 0 to day 90 (V=18, P=0.18).

Lameness was correlated positively with total lesion scores (r_s = 0.89, P<0.0001). TPS increased as lameness scores increased (r_s = 0.51, P<0.0001) and as total lesion scores increased the TPS also increased (r_s = 0.50, P<0.0001).

3.2 Mastitis

The facial expression scale showed good accuracy at correctly identifying diseased sheep from control sheep with AUC of 0.80. Table 1 shows the sensitivity and specificity of each TPS for mastitis sheep on day 0. Table 2 gives details on the correlation between facial areas and TPS. There were no main effects of breed (P=0.22) or farm (P=0.31) on TPS. TPS was affected by a time*treatment interaction (P=0.02). Sheep in group MAN had a higher TPS score (4 ± 0.54) than did sheep in group MC (2 ± 0.47) on day 0 ($F_{(1, 20)}$ = 7.52, P=0.01). There were no significant differences in TPS between treatment groups for day 7 (χ^2=0.01, df=1, P=0.92) and day 42 (χ^2 = 0.03, df=1, P=0.87) (Fig. 3). Sheep in group MAN had a significant decrease in their facial expression score between days 0 and day 7 (t=-2.15, df=11, P=0.05) and between days 0 and days 42 (t=-9, df=11, P<0.001). The TPS did not change between day 7 and day 42 for sheep in group MAN (t=-1.61, df=11, P=0.14) and did not change for sheep in group MC between days 0 and day 7 (t=1.03, df=9, P=0.33), days 0 and day 42 (t=0.133, df=9, P=0.90) and days 7 and 42 (t=-0.58, df=9, P=0.58).

3.3 Five trained observers

The average accuracy of the global pain assessment was 67%, with individual accuracy ranging from 60% to 75%. Of the errors, false positives (26.3%) were more common than false negatives (6.3%). The TPS had a high accuracy in relation to lameness with an AUC of 0.84. Table 1 shows the sensitivity and specificity of each level of the TPS given by observers. Table 2 gives details on the correlation between facial areas and TPS. There was a high inter-rater reliability with an overall intra-class correlation (ICC) value of 0.86. Each of the facial areas assessed also showed high (orbital tightening, 0.90; cheek tightening, 0.82; abnormal ear position, 0.85) to medium ICC values (abnormal lip and jaw profile, 0.63; and abnormal nose position, 0.65). The five facial areas were scored easily by all participants as demonstrated by the percentage of “not able to score” ranging from 0% for orbital tightening to 12% for cheek tightening.

There was a main effect of breed (P=0.02); however, when performing contrasts, there were no significant differences identified between breeds (P>0.05). There were no significant effects of gender (P=0.46) or farm (P=0.71) on TPS. Time, treatment and time*treatment interaction had an effect on TPS (P=0.001, P=0.02, P=0.003, respectively). There were differences between treatment groups on day 0 ($F_{(2,27)}$ = 11.33, P=0.0003). Sheep in group FOA (4.78 ± 0.49) had a higher TPS than sheep in group FOC (2.70 ± 0.30) (Tukey post-hoc, P=0.007). Sheep in group FOAN (5.45 ± 0.47) also had a higher TPS than sheep in group FOC (Tukey post-Hoc, P=0.0002). Sheep in group FOA and group FOAN did not differ in TPS on day 0. There were no differences in TPS on day 90 between treatment groups (χ^2 = 1, df = 2, P=0.61). Participants did not score sheep in group FOC differently on day 0 compared to day 90 (t=0.33, df=9, P=0.75). Sheep in group FOA had a lower TPS on day 90 compared to day 0 (V=4, P=0.05) as did sheep in group FOAN (t = -5.49, df=10, P=0.0003) (Fig. 4).

Lameness was correlated positively with total lesion scores (r_s = 0.82, P<0.0001). TPS increased as lameness scores increased (r_s = 0.56, P<0.0001) and as total lesion scores increased the TPS also increased (r_s = 0.54, P<0.0001).

4. Discussion

The SPFES developed for this study showed a high degree of accuracy, differentiating between lame and non-lame sheep correctly, through identifying changes in the facial expressions according to their level of lameness. These changes in facial expression are similar to those described in other species with respect to pain (Dalla Costa et al., 2014; Keating et al., 2012; Leach et al., 2012; Sotocinal et al., 2011). Importantly, there were no changes in the facial expression of non-lame sheep. Sheep that had been suffering from footrot showed high total pain scores that decreased as they recovered. Total pain scores were positively related to both the total lesion scores and the lameness scores, providing further evidence for pain in sheep to be both a sensory and emotional experience. The positive correlation between the level of lameness and severity of footrot lesion observed in our study confirms our choice of model and is in agreement with other studies (Dolan et al., 2003; Kaler et al., 2011).

Although we could not differentiate between groups FA and FAN at either time point the provision of analgesic treatment to sheep with footrot at the time of disease diagnosis appeared to reduce the total pain score over the 90 day observation period further, compared with sheep that only received an antibiotic. This result was also noted by the five trained observers whereby the FOAN group had a larger decrease in their scores between day 0 and day 90 compared with the FOA group. This decrease in total pain score supports the need to manage pain in sheep with this disease. It is possible that the use of a non-steroidal anti-inflammatory drug may have reduced the effects of potential "wind-up" from persistent excitation of the nociceptors involved with the footrot lesions (Stein, 2013; Viking Höglund and Frendin, 2002); however, further investigation is required. The reduction in pain could have allowed the sheep to recover more efficiently and resume normal activity before sheep that had not received this additional treatment. Treatment was given to sheep on day 0 after the photographs had been taken and so no effect of analgesic would have been occurring at the time of photography. Any effect of analgesia would have been detectable up to 72 hours after the administration of the analgesic as suggested by its elimination half-life (Shukla et al., 2007). In future studies, it would be beneficial to monitor the changes of facial expression over this time period.

The high level of specificity for a total pain score above 5 for each of the diseases indicates that a sheep given this score or above are unlikely to be a false positive. Sheep scoring a total pain score above 5 are therefore likely to benefit from the administration of pain relief. Although the sensitivity of the test is low, meaning that some of the diseased animals may go undetected below a TPS of 5, the overall accuracy of the test is high. It is preferable for a test such as this to have a higher specificity rate where sheep reaching a high pain score are unlikely to be negative for the painful disease.

Total facial expression scores at day 90 were not zero. It is possible that hyperalgesia remained a contributing factor within our study population. Ley et al. (1995) also found sheep previously diagnosed with footrot were still showing an increased response to mechanical stimulation compared to control sheep three months after they had seemingly recovered. Control sheep were also not scored as zero on day 0 or day 90, a finding observed in other studies using facial expression as a pain scoring system (Dalla Costa et al., 2014; Keating et al., 2012). There are several possible explanations for this. Control sheep may have had a previous episode of footrot that was not evident at the time of clinical examination on day 0 and the associated hyperalgesia may have still been present. Additionally, facial expression may change due to other affective states such as fear and stress, which can both be related to pain. The development of facial expression scales to help identify other affective states, both positive and negative, would be beneficial.

The SPFES also showed a high degree of accuracy in correctly differentiating between sheep with mastitis and controls. The total pain scores of sheep with mastitis were higher than control sheep on day 0 and decreased rapidly in response to treatment by day 7. Facial expressions in sheep with mastitis did not change significantly from day 7 to day 42 suggesting that the provision of a non-steroidal anti-inflammatory drug as well as antibiotic treatment reduced the associated pain substantially by day 7. Importantly there were no changes in the facial expression of sheep that acted as controls across time. These results provide further evidence that the SPFES is accurate at identifying changes in facial expression that suggest pain in sheep associated with disease.

The results from the five observers are in line with those given by the more experienced scorer and demonstrates that the provision of basic training allowed for the effective use of the SPFES to be used accurately and reliably. In addition, the similarity in results from the main observer and the treatment and session blinded observers, provides evidence that bias was unlikely to be present in the main observer. The total pain scores given by the observers correctly identified lame and non-lame sheep, giving higher scores to lame sheep compared to control sheep on day 0. The observers also scored sheep at day 90 as low and similar between groups. Observers' scores also correlated positively with both the lameness and the lesions scores, supporting the use of the SPFES in identifying pain. The global pain assessment given by observers was lower (67%) than that of other "Grimace Scales" (97% for the Mouse Grimace Scale, (Langford et al., 2010), 84% for the Rabbit Grimace Scale (Keating et al., 2012), but similar to the Horse Grimace Score (73.3%) (Dalla Costa et al., 2014). Scorers were readily able to identify pain when present, but were cautious in diagnosing absence of pain. In terms on animal welfare, this is the preferable result. However, the accuracy of the scale improved (up to 84%) when scores given to each area were combined to give a total pain score. This provides support for the use of the SPFES at identifying pain in sheep in relation to disease, rather than giving a global assessment. The increase in objectivity through the use of the scale potential helps to remove any fear of not identifying a sheep in pain correctly.

The SPFES scale is reliable between scorers with an overall inter-rater reliability score of 0.86, and there was high consistency in scores given to the orbital area, the cheek area and ear positioning, similar findings to others (Keating et al., 2012; Sotocinal et al., 2011). The lip and jaw profile along with the nostril and philtrum positioning were less reliable between scorers, a result also noticed for the Horse Grimace Scale (Dalla Costa et al., 2014). The nostril and philtrum position also did not correlate well with the other areas of the face. This is likely due to the way in which images were captured. Images for this study were taken as individual photographs rather than still images captured from video footage. Low image quality and photographs taken at poor angles were avoided wherever possible; however, there may still be possible negative impacts on effective scoring, a problem noted within other validation studies of facial expression scales in animals (Dalla Costa et al., 2014; Keating et al., 2012; Langford et al., 2010).

Farm and gender did not have any significant effect on the total pain score across treatment group and time supporting its use as an on farm assessment tool. Breed was only noted to have a significant main effect on total pain scores in the trained observer group; however, on further investigation there were no significant differences between breeds found. The anatomical differences between some breeds of sheep, as well as different colours of the face, may have made it difficult for some observer's to score areas effectively. However, the muscle groups involved in facial expression will be the same in each breed and so the changes in facial areas will be the same movement (see Fig. 1 abnormal ear position for an example of this). Facial areas were well correlated with the total pain score across diseases. There are some areas of the face that correlate with each other well; orbital tightening, abnormal ear position and abnormal lip and jaw profile. Sheep suffering with mastitis had several areas of the face that were not well correlated with each other. This could be due to the smaller sample size for the mastitis group, or it could be a factor of the disease state. The systemic nature of mastitis is more likely to leave sheep dehydrated and therefore some areas of the face may be affected by this, such as the orbital and cheek area may appear sunken. It is important that a full assessment of any animal is carried out if disease is suspected and taken into account when scoring the animals. Changes in the facial expression occur during other activities such as blinking or chewing which can change the appearance of the orbital area and cheek area respectively. Every effort was made to eliminate photographs that may have been taken during these activities; however, using the SPFES to score animals 'live' rather than using still images would resolve many of these problems. Fluctuation in pain will also result in a fluctuating facial expression. Scoring animals live would identify these fluctuations through the changes in facial expression and may lead to a better ability at assessing the intensity of the pain experienced. Future trials for scoring animals live after initial training are currently being planned to further investigate the use of the SPFES on farm.

5. Conclusion

The major challenge for pain research is being able to assess the emotional side of pain (Flecknell et al., 2011). Facial expression as a pain scoring method offers the potential to start to understand this side of animal pain (Kunz et al., 2012, 2009) and the results from the current study support this. At present, the SPFES has been assessed using footrot as the clinical model and successfully applied to mastitis, a disease causing acute pain in sheep. It is likely that the scale can be used for other conditions that are suspected of being painful, such as pregnancy toxaemia where the administration of a non-steroidal anti-inflammatory is known to aid recovery (Zamir et al., 2009). The current scale provides an accurate and reliable method to recognise and assess pain in sheep. It is also doubles as a training tool for veterinarians and farmers to learn more about changes in the facial expression of sheep when they are likely to be suffering from pain. Such a tool is likely to improve an observer's ability to quantify pain in animals and allow observers to discriminate between different pain states independent of disease status, as well as detect the effectiveness of pain relief. Prompt recognition of pain through the use of the scale will enable farmers and veterinarians to treat and manage their flocks better, reducing the impact of pain on their sheep, thus improving welfare and production. It is important to stress that the scale should be used as part of other measures of pain and not as a standalone assessment. The provision of the sensitivity and specificity of the scales at each level of pain will aid scorers in their decision of when to intervene with pain management; something that is often missing from such scales. This will lead to better management of flocks, leading to better production values and higher welfare for the sheep.

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Conflict of interests

There are no conflicts of interests for any of the authors.

Author Contributions

Gathered data on farms: KMM, CJR, MC. Analysed data: KMM and MH. Interpreted results and drafted manuscript: KMM. Read, edited and approved the manuscript: KMM, CJR, MC, ML, MH, FCC.

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Table 1. The sensitivity and 1 - specificity of total facial expression scores for each disease for different positive cut-off points, as scored by the experienced observer (footrot and mastitis) and by the five trained observers (five observers – footrot). A high sensitivity value indicates a high percentage of sheep identified as being positive for the disease, if the pain score is greater than or equal to the total pain score value listed. A low 1-specificity value indicates a high percentage of sheep correctly identified as not having the disease if the pain score is greater than or equal to the total pain score. *Note, the 1-specificity value of 0.000 indicates all sheep that did not have the disease were not given a total pain score above this level, i.e. they were correctly identified as not having the disease.

Total pain score: positive if greater than or equal to	Footrot		Mastitis		Five observers - footrot	
	Sensitivity	1 - Specificity	Sensitivity	1 - Specificity	Sensitivity	1 - Specificity
1.5	0.927	0.459	1.000	0.600	1.000	0.791
2.5	0.829	0.311	0.667	0.400	0.941	0.581
3.5	0.512	0.180	0.667	0.200	0.882	0.395
4.5	0.293	0.016	0.333	0.000*	0.647	0.163
5.5	0.171	0.000*	0.167	0.000	0.412	0.047
6.5	0.049	0.000	-	0.000	0.176	0.000*
7.5	0.024	0.000	0.083	0.000	0.118	0.000
9	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. Correlations between each areas of the face and the total pain score from sheep scored by KM with footrot represented in the top row, sheep scored by the five trained observers in the middle row and sheep with mastitis in the bottom row. *P<0.05, **P<0.01, ***P<0.001.

	Orbital tightening	Cheek (masseter) tightening	Abnormal ear position	Abnormal lip and jaw profile	Abnormal nostril and philtrum shape	Total Pain Score (TPS)
Orbital tightening	-	0.32***	0.41***	0.26**	0.20*	0.61***
		0.51***	0.52***	0.42***	0.37***	0.73***
		0.09	0.37**	0.29*	0.25	0.66***
Cheek (masseter) tightening	0.32***	-	0.18	0.36***	0.32***	0.60***
	0.51***		0.45***	0.45***	0.47***	0.75***
	0.09		0.18	0.26	0.01	0.45***
Abnormal ear position	0.41***	0.18	-	0.34***	0.20*	0.64***
	0.52***	0.45***		0.52***	0.50***	0.78***
	0.37**	0.18		0.37**	0.02	0.59***
Abnormal lip and jaw profile	0.26**	0.36***	0.34***	-	0.36***	0.47***
	0.42***	0.45***	0.52***		0.63***	0.79***
	0.29*	0.26	0.37**		0.24	0.73***
Abnormal nostril and philtrum shape	0.20*	0.32***	0.20*	0.36***	-	0.62***
	0.37***	0.47***	0.50***	0.63***		0.77***
	0.25	0.01	0.02	0.24		0.55***
Total Pain Score (TPS)	0.61***	0.60***	0.64***	0.47***	0.62***	-
	0.73***	0.75***	0.78***	0.79***	0.77***	
	0.66***	0.45***	0.59***	0.73***	0.55***	

Orbital tightening

Not present = 0

Partially present = 1

Present = 2

There is a closing of the palpebral fissure by the eyelids and a narrowing of the eye aperture. If the eye closes more than half way it should be scored as present (2).

Cheek (masseter muscle) tightening

Not present = 0

Partially present = 1

Present = 2

There is a more convex shaping to the cheek in the area of the masseter muscle and the zygomatic arch as tension increases.

Abnormal ear position (front)

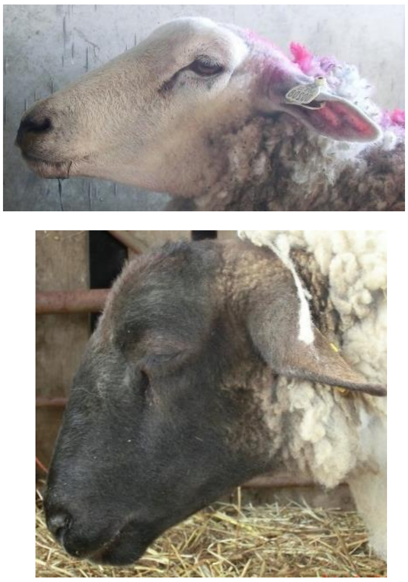
Not present = 0

Partially present = 1

Present = 2

The ears become fully rotated ventrally and caudally and the inner pinna of the ear becomes less visible. Note: Baseline (not present) ear carriage varies between breeds; however, changes in ear position are the same.

Abnormal ear position (side)



Not present = 0	Partially present = 1	Present = 2

The ears become fully rotated ventrally and caudally and the inner pinna of the ear becomes less visible. Note: Baseline (not present) ear carriage varies between breeds; however, changes in ear position are the same.

Abnormal lip and jaw profile

Not present = 0	Partially present = 1	Present = 2

The lower lip is drawn back caudally and the jaw profile appears straight to concave. The chin and jaw line are straightened. The lip line to the commissure of the mouth is straight or even rotated ventrally.

Abnormal nostril and philtrum shape		
 **Not present = 0**	 **Partially present = 1**	 **Present = 2**
As the philtrum is shortened and narrowed increasing a concave appearance of the upper lip profile, a ‘V’ shape between nostril apertures is present. The V shape is mimicked in the surrounding nose area.		

Fig. 1. The Sheep Pain Facial Expression Scale (SPFES). The Sheep Pain Facial Expression Scale with images and descriptors of each facial area. Each facial area is scored according to whether it is not present (score of 0), partially present (score of 1) and present (score of 2). Note: not every facial area will be present when scoring the expression. Some areas may be expressed at the highest level, whilst others are not present, in the same sheep.

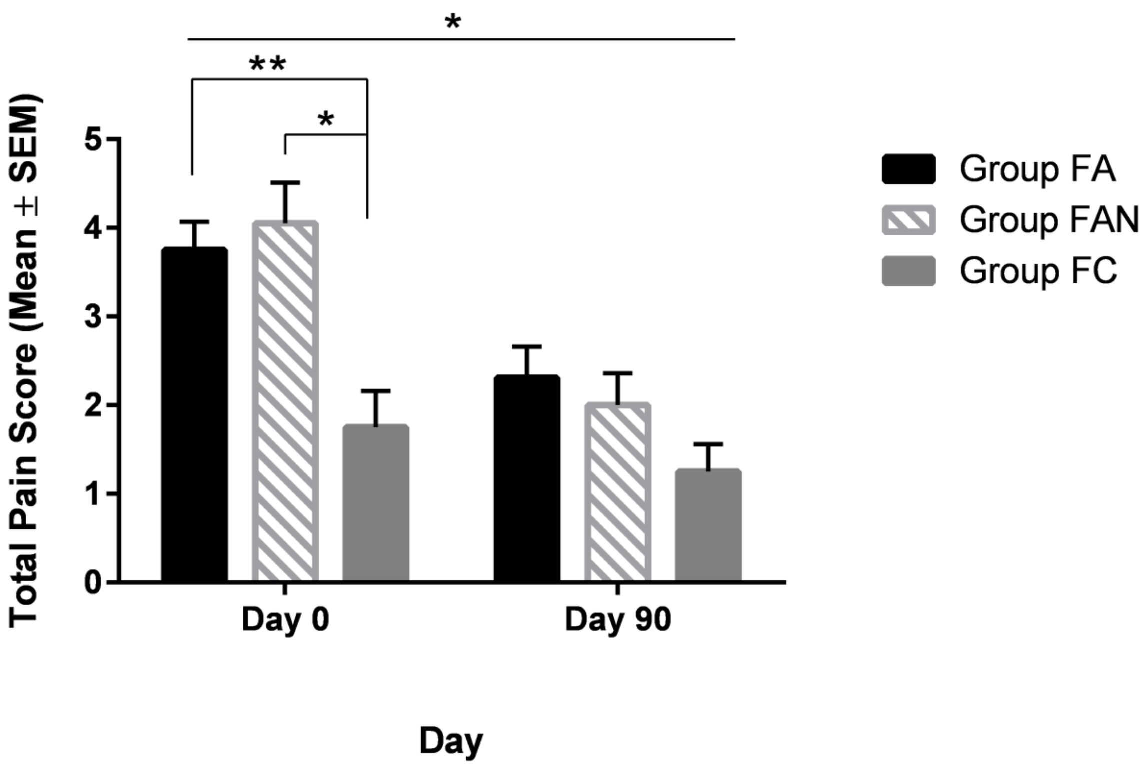


Fig. 2. Total facial expression pain score (mean ± SEM) of sheep treated for footrot with systemic antibiotics (FA), with antibiotics plus a non-steroidal anti-inflammatory drug (FAN) and control sheep (FC), as scored on day 0 and day 90 by an experienced observer. *P<0.05, ** P<0.01

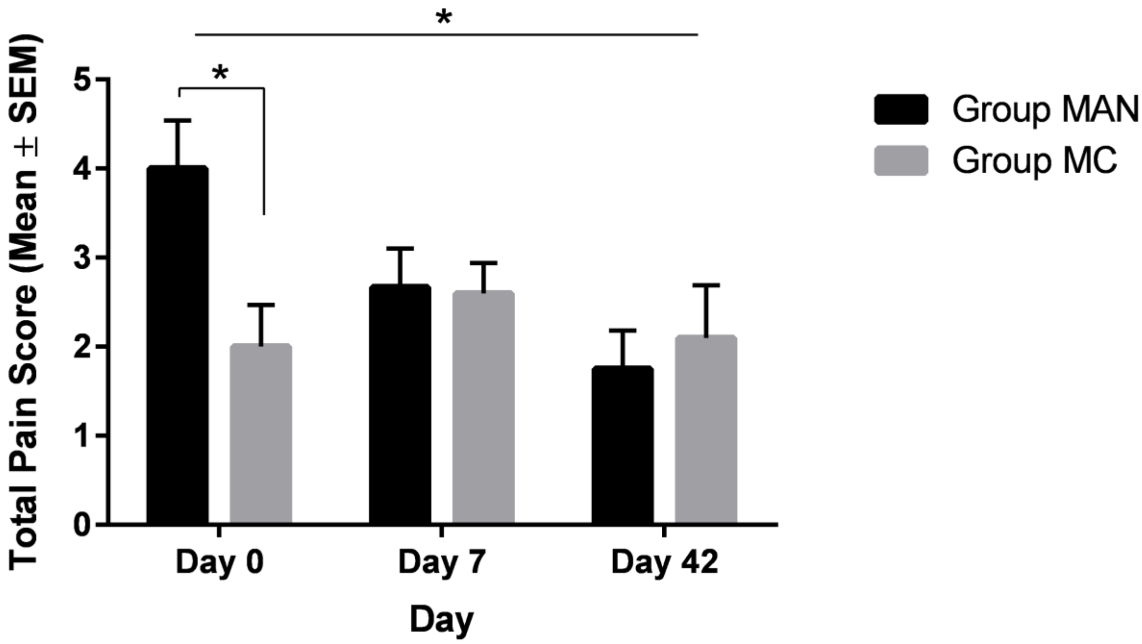


Fig. 3. Total facial expression pain score (mean ± SEM) of sheep treated for mastitis with systemic antibiotics and a non-steroidal anti-inflammatory drug (MAN) and control sheep (MC), as scored on day 0, day 7 and day 42 by an experienced observer. * P<0.05.

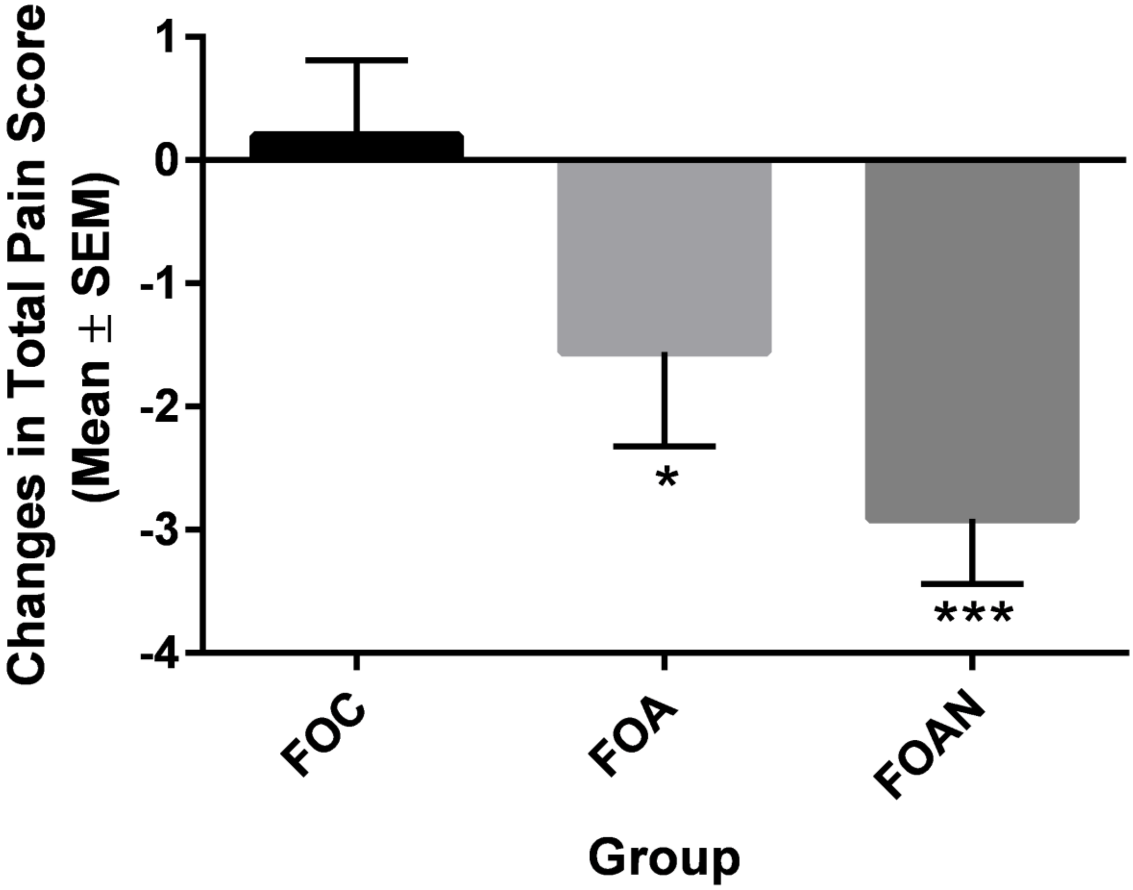


Fig. 4. Changes in facial expression total pain score (mean ± SEM) from day 0 to day 90 of sheep treated for footrot with systemic antibiotics (FOA), with antibiotics plus a non-steroidal anti-inflammatory drug (FOAN) and control sheep (FOC), as scored by five trained observers. * $P<0.05$, *** $P<0.001$.