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Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia.

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Abstract:

Caustic paste is frequently used for disbudding young female dairy calves. Nerve blocking may not be completely effective after such chemical tissue damage. Regional anaesthesia, together with a non-steroidal-anti-inflammatory drug (NSAID), was shown to reduce plasma cortisol in calves disbudded using caustic paste. To find out whether pre-emptive NSAID alone could control pain or whether NSAID reduces cortisol response by a mechanism other than by pain control, we compared cortisol levels and behaviour of 10 chemically-disbudded calves treated with IV flunixin-meglumine, five of which were injected at 5 minutes (F0) and five injected at 60 minutes before dehorning (F1), with 5 sham-dehorned (ND) and 5 non-treated chemically-disbudded animals (CD).

There was a higher ($p<0.001$) cortisol level in both NSAID-treated groups compared with ND at 1 hour after disbudding, but no differences from control animals (CD). Behaviour analysis showed these same differences up to three hours post-disbudding.

We conclude that pre-emptive analgesia treatment by itself is not effective in controlling pain and does not prevent blood cortisol increase after disbudding of calves with caustic paste.

Keywords: dehorning calves analgesia cortisol behaviour

Introduction:

The disbudding of female calves on dairy farms is a mutilation but is used because of the potential welfare and security advantages for animals and humans. It is approved in European legislation under certain conditions (Council Directive 91/629/EEC of 19 November 1991, amended by Council Directive 97/2/EC).

Plasma cortisol and certain disturbed behaviours (e.g. certain vocalisations, changes in posture, changes in locomotor activity, head-shaking, stamping, kicking, scratching or rubbing), are considered to be good indicators of acute pain such as that which probably occurs after dehorning (Broom and Johnson, 2000; Molony. 1997).

There are three main methods for dehorning young calves: hot iron (cauterisation), amputation (scoop, guillotine and others) and chemical (caustic paste, usually with a very strong base like sodium hydroxide or calcium hydroxide). A recent survey of cattle veterinary surgeons showed that caustic paste was the most frequently used method (Stilwell, unpublished). The reasons for this may be: (1) it is quickly and easily performed by farmers (2) the reduced degree of resistance by the calf at handling gives the impression that it is a less painful procedure.

However, it has been demonstrated that caustic paste causes acute pain with a significant rise in plasma cortisol for at least one hour and the performing of pain behaviours (e.g. head-shaking, scratching, rising and lying frequently) for more than three hours (Stilwell *et al*, 2004a). These effects are even more prolonged when animals are directly exposed to sunlight (Stilwell, personal observations).

Strong alkali cause liquefactive necrosis, resulting in saponification of fats and denaturation of proteins, which allows deeper penetration of the chemical. Alkalis tend to penetrate deeper and cause worse burns than acids (Hettiaratchy *et al*, 2004). With alkali burns, tissue damage continues to increase as long as the active chemical is in contact with the tissue (Yano *et al*, 1993). These authors showed that after using sodium hydroxide to inflict alkaline injury on rats, the subcutaneous tissue pH reached its peak value at the 32nd minute and had not recovered to the pre-experimental level by the 90th minute.

Sodium hydroxide, which is commonly used for calf disbudding, is a very strong (pH 14) and corrosive alkali and causes pain that is described by humans as an "itching pain" or "marked pain" (Ma Bing *et al*, 2007) or sometimes as a chronic and severe pain (Kumbhat *et al*, 2004).

Very little is known about the pain caused by caustic burns on animals and only a few studies have looked at the ways to control the distress of calves disbudded by this method (Vickers *et al*, 2005; Stilwell *et al*, 2004b,; Morrisse *et al*, 1995). Several studies have demonstrated the efficacy of using a NSAID together with local anaesthesia in controlling pain after hot iron or amputation dehorning (Stilwell *et al*, 2004b; Stafford et al, 2003; Sutherland *et al*, 2002; Faulkner *et al*, 2000; McMeekan *et al*, 1998). Morisse et al (1995), on the other hand, suggested that local anaesthesia was not very efficient in reducing pain for the first few hours after caustic paste disbudding. The lack of efficacy of the local anaesthesia was also evident from the behaviour of calves disbudded with caustic paste after sedation with xylazine (Vickers *et al*, 2005). Our previous studies (Stilwell *et al*, 2004b) have shown that local anaesthesia (lidocaine) associated with an analgesic drug (flunixin-meglumine) does eliminate blood cortisol rise and pain related behaviours after chemical disbudding, but it did not show whether this effect was due to the combined use or to anti-inflammatory drug only.

Only one study (McMeekan *et al,* 1998) has looked at the effect of a NSAID on its own (ketoprofen 20 minutes before amputation dehorning). The authors only measured plasma cortisol and the results showed that during the first 1.30 hours after dehorning the mean cortisol concentrations did not differ significantly from non-treated scoop-dehorned calves.

The effects of chemical tissue damage on nociceptors are not fully understood and the role of NSAID as a cyclo-oxygenase (COX) inhibitor may not be the most important in controlling this kind of pain. In humans, pain from chemical burns is usually controlled with opioid drugs like tramadol hydrochloride (Ma Bing *et al*, 2007) or methadone (Altier *et al*, 2001).

All known NSAID exert their therapeutic effect mainly by COX inhibition but some also possess other actions at the molecular level, both peripheral and central (Lee *et al*, 2004). It is known that NSAID exert their anti-pyretic effect by inhibiting prostaglandins at the hypothalamus. The possibility that NSAID may influence the functioning of the Hypothalamic-Pituitary-Adrenal axis (HPA) during a stress/pain response to chemical burn, by means of effects other than analgesia, is not known.

Flunixin-meglumine is a NSAID considered as having a powerful analgesic effect and a half-life of approximately 7 hours in calves (Landoni *et al*, 1995).

This study was designed to answer two questions. Firstly, if local anaesthesia is not very efficient in controlling pain after chemical disbudding but local anaesthesia associated with NSAID is effective, can pain be prevented with only pre-emptive use of flunixin-meglumine? Secondly, does NSAID reduce cortisol levels, after paste disbudding by means of mechanisms other than its analgesic effect? We study this by comparing the effect of treatment on blood cortisol and pain-related behaviours.

Material and Methods

Experimental procedures

The study was carried out on a 700 adult cow dairy farm, 50 kilometres north of Lisbon, Portugal.

Twenty, 10 to 40 days of age (no difference in age between groups), female Holstein-Friesian calves were included in this study. The calves were kept in a group pen which consisted of a straw-bedded lying area and a solid-floor feeding area. An outside exercise area was usually available but was closed for the duration of the study. Animals were fed whole milk and concentrate from two computer-controlled feeding stations.

The calves were allocated randomly (5 numbers taken from a bag) to each treatment group. The non-steroidal-anti-inflammatory-drug (NSAID) used in this study was flunixin-meglumine (Finadyne, Schering-Plough ®). Treated calves were injected intravenously (jugular vein) with 4 ml (± 0.2 mg/kg) one hour before disbudding (Group F1; mean age 27 ±12 days) or 5 minutes before disbudding (Group F0; mean age 25 ±12 days). Non-dehorned (Group ND; 30 ±6 days) animals were injected IV with 4 ml of saline solution after first blood sampling (5 minutes before disbudding). The control group calves (Group CD; mean age 24 ±10) were chemical disbudded with no treatment. The study was carried out in two different days but the pen, time of day, weather and stockman performing the disbudding, were exactly the same for all calves.

Five minutes after first blood collection, calves were forced to lie down, hair was clipped around horn buds and the caustic paste (SH-Plus® - Sodium Hydroxide) was applied with a spatula (following the normal procedure at this farm). ND animals were handled in the same way (including hair clipping) but instead of paste the horn buds were rubbed with an obstetric gel (VetTop Gel ®) for the equivalent time.

Animals were coloured-marked on both sides with a randomly chosen number for easier identification when behaviour was assessed. The observer was an experienced veterinary surgeon blind to the treatments.

Blood sampling (7ml) was into a heparinised tube by left jugular venipucture at 5 minutes before disbudding and at 1, 3, 6 and 24 hours after disbudding. Blood was kept in ice then centrifuged and frozen (-20ºC). Previous studies (Stilwell and Lima 2004) showed that careful blood-sampling did not elicit an increase in cortisol in calf plasma.

Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using commercial kits (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). The inter-assay coefficients of variation for cortisol were 5.5% for the level of 1μg/dL and 1.9% for the level of 5 μg/dL.

Five distress-reactions were looked for while the calf was lying and the disbudding procedure was carried out: trying to stand on front legs, extending hind legs, head shaking, vocalisation and open mouth with no sound. Individual grading could be from 0 (none of the reactions observed) to 5 (all the reactions recorded).

Behaviour observations after disbudding were made for periods of 15 minutes, at 15 minutes, 1, 3, 6 and 24 hours. We recorded the following behaviours that have been previously used to evaluate pain after disbudding (Vickers *et al*, 2005; Stilwell *et al*, 2004b,; Grøndahl-Nielsen *et al*, 1999; Morrisse *et al*, 1995):

a) head-shake, b) ear flick, c) hind-limb scratching head or head rubbing against objects, c) quick transition from standing to lying and back to standing, d) inert lying (lying with muzzle on flank and no reaction to surroundings).

All behaviours could be recorded more than once for each calf during each 15 minute period, with the exception of “inert lying” that could only be registered once for each observational period.

Statistical analysis

Distributions of these variables were shown by Levene and Shapiro-Wilks tests to be non-normal, so non-parametric analyses were used. Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between the four groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance. *P*-values less than 0.05 were considered significant.

Results:

The cortisol results (Table 1) showed no difference between groups in base-line values ($p = 0.55$). At one hour after dehorning the two NSAID-treated groups showed an increase in cortisol compared with the baseline values ($p < 0.05$) and compared with the ND group ($p < 0.001$). The values were equal to the ones shown for dehorned but not treated animals (CD). The non-dehorned calves showed a lower cortisol level at +24 hours compared with +6 hours.

The analysis of the behaviour during the disbudding procedure (Table 2) showed very few reactions (head-shaking and hind limb extension) to the restraining and paste/gel application in any of the groups of calves. No calf vocalised. On the other hand there was a significant difference in total pain-behaviour incidence (Table 3) between each of the disbudded groups and the ND group at 15 minutes and 1 hour. The difference between F0 and F1 compared with ND was still significant at 3 hours. There was no difference in behaviour frequency at any time, between any of the disbudded groups. All disbudded groups showed a significant increase in disturbed behaviours at 15 minutes, 1 hour and 3 hours compared with behaviours shown at 6 or 24 hours. Group F1 was the only group that showed higher frequency of disturbed behaviours at 15 minutes compared with all other periods of observation.

Discussion and conclusions:

All four groups involved in this study showed similar cortisol base-line levels. These animals were of similar ages, had a common background and their gentle handling did not cause them distress.

After disbudding the plasma cortisol concentrations were highest at 1 hour in all disbudded animals including those treated with NSAID. Other studies found a similar increase in chemically disbudded non-treated animals (Stilwell *et al*, 2004a; Morisse *et al*, 1995). The cortisol results of our study, when we take account of the context, as proposed by Broom and Johnson (2000), show that caustic paste disbudding causes poor welfare in calves and that analgesic treatment, even if given in a pre-emptive way, is not sufficient to prevent the cortisol rise in disbudded but non-treated animals.

The high cortisol values at + 6 hours (time of day: 16.00 h) compared with -5 and 24 hours (time of day: both 10.00 h) in the ND group may be due to circadian variation or some husbandry factor not identified.

The small level of reactions shown by all the calves to the handling and actual disbudding can be explained as follows: these animals are used to the presence and even contact with herdspersons; their size and strength are still easily subdued by an experienced operator; pain after tissue damage by chemicals only starts a few minutes after application, as explained by (Choinière *et al*, 1989). The fact that no calf vocalised during the procedure is also a sign of reduced distress because young animals usually vocalise when severe fear or pain is elicited (Watts *et al*, 2000).

The behaviour observations support the idea of distress caused by disbudding. At 15 minutes, all disbudded calves showed a very significant incidence of pro-active pain-related behaviours. These may alleviate the "itching pain" sensation (Ma Bing *et al*, 2007). Other behaviours also observed, but not recorded, included backing and even falling after shaking the head vigorously. At one hour after disbudding the number of disturbed behaviours was still high but the incidence was reduced. At this time and at three hours, some animals changed from a pro-active behaviour to a passive one (inert lying). This behaviour, recorded in other studies with lambs after castration and described as the time during which it was difficult to elicit any evidence of conscious awareness (Molony *et al*, 1993), might be stress-induced and so an important indicator of an aversive experience (Gregory, 2004). Two of the calves that showed this behaviour at 3 hours also had the highest cortisol level (110.26 and 111.05 nmol/L). Lane (2006) suggests that helplessness in animals is perhaps the closest correlate to a depressive state and very high levels of glucocorticoids have been found in animals suffering from this condition (Gregory, 2004; Sumida *et al*, 2004). This behaviour was not observed in our previous studies with animals dehorned by scoop or hot-iron (Stilwell *et al*, 2004a). We suggest that this could be because of the type of pain or the age of the calves (younger in the present study). The "inert lying" behaviour also has another effect: the animal does not perform as many behaviours and so reduces the group total count for that time period.

The study by Vickers *et al* (2004) looked at behaviours (head-rub, head-shake and transition), following caustic paste dehorning but used sedated animals, so the behaviours during the first hour or two might have been obscured. Even so, that study showed similar results to ours: a higher level of the three behaviours in paste-disbudded animals compared with sham-disbudded ones, during the first four hours.

Contrary to what Morisse t al (1995) suggest, we found a difference in the duration of the cortisol (1 hour) and the behaviour (3 hours) rise, showing that pain evaluation should use these two kinds of indicators.

Previous studies (Stilwell et al, 2004b) have shown that flunixin-meglumine in combination with lidocaine does reduce cortisol response to chemical disbudding, but this could be a direct effect of the NSAID on cortisol production and not necessarily because of its analgesic effect. With the present study we showed that flunixin-meglumine, as a sole treatment, does not prevent cortisol rise after a painful experience.

We concluded that analgesia with flunixin-meglumine, even if administered in a pre-emptive way, is not sufficient to reduce cortisol release and to control acute pain caused by the chemical tissue damage during disbudding of young calves.

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