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ASSESSING PAIN IN CALVES AFTER DISBUDDING

Effect of Hot-Iron Disbudding on Behaviour and Plasma Cortisol of Calves Sedated with Xylazine.

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ABSTRACT

We investigated physiological and behavioural responses at 10, 25, 40 and 60 minutes after hot-iron or sham disbudding of 41 Holstein calves aged between 29 and 41 days. Animals were randomly divided into 4 groups: disbudded after *i/m* xylazine, disbudded after *i/m* xylazine and cornual nerve blocking with lidocaine, sham-disbudded after *i/m* xylazine and cornual nerve blocking with lidocaine, sham-disbudded after *i/m* saline and lidocaine. The 3 groups treated with xylazine showed higher plasma cortisol concentrations than saline-treated animals at all times including before the procedure (-5 min). There were no cortisol inter-group differences in xylazine-treated groups at any time. Only xylazine-alone disbudded calves showed an increase in cortisol at +10min compared with base-line.

Sham-disbudded calves treated with xylazine showed a lower cortisol value at +60 min compared with -5, +10 and +25 min. The degree of struggling during disbudding was higher in the calves disbudded after xylazine than in all other groups and animals sham-disbudded with no xylazine struggled more than sham-disbudded with xylazine. Disbudded calves treated only with xylazine showed more pain-related behaviours at 10 and 40 min than all the other groups. At 60 min all groups given xylazine showed more behaviours than non-treated animals. Ear-flick was the only behaviour for which incidence differed between groups.

We conclude that xylazine given intramuscularly to young calves, causes a rise in cortisol that may disguise increases due to pain-related distress during at least the first hour after hot-iron disbudding. Behaviour analysis is also confused by the high incidence of some behaviours shown by calves when sedated or when awakening from sedation. Only struggling during the procedure and the incidence of ear-flicks immediately after disbudding could be considered signs of pain caused by the tissue damage.

KEY WORDS: hot-iron disbudding, pain, plasma cortisol, behaviour

INTRODUCTION:

Disbudding of young female dairy calves is a common procedure in farms. The two methods most commonly used are: caustic paste (chemical burn) and hot-iron (thermo-cautery). Hot-iron disbudding is done by applying the device, electric or butane-gas heated to over 600°C, over the horn bud destroying the growing tissue at its base. This method is performed when horn-buds are evident by palpation corresponding to the age of 4 to 8 weeks. Even at this age hot-iron disbudding causes severe pain-related distress that is demonstrated by significant plasma cortisol rise (Boandl *et al*, 1989; Morisse *et al*, 1995; Graf *et al*, 1999) and behavioural changes (Faulkner *et al*, 2000; Vickers *et al*, 2005; Doherty *et al*, 2007). Local anaesthesia (cornual nerve blocking) has been shown to delay the pain responses for at least 2 hours (Graf *et al*, 1999; Grøndahl-Nielsen *et al*, 1999; Faulkner *et al*, 2000; Vickers *et al*, 2005; Doherty *et al*, 2007). All these studies show that applying the hot-iron with no anaesthesia causes pain so that significant physical restraint is necessary to carry out the procedure.

Xylazine is a α 2-adrenoceptor agonist particularly active in cattle (Törneke *et al*, 2003) causing central nervous system depression, ranging from mild sedation to recumbency, and a decrease in heart rate, respiratory exchange ratio and plasma adrenaline concentrations (Campbell *et al*, 1979; Scholtysik *et al*, 1998). Alpha2-adrenergic agonists also inhibit transmission of nociceptive stimuli in the dorsal horn of the spinal cord by simulating the effect of noradrenaline released by inhibitory descending pathways (Sullivan *et al*, 1987; Petovaara *et al*, 1990). It is a drug commonly used for hot-iron disbudding (Mish *et al*,

2008; Faulkner *et al*, 2000; Stilwell, personal observations) because it facilitates handling and because it reduces responses after the procedure, giving the idea that distress is low. By giving xylazine the hot-iron disbudding may be performed by just one person with security advantages for the operator and the animal. It may also reduce the time needed for the complete destruction of the horn tissue.

Changes in concentrations of circulating glucocorticoids, reflecting the activation of the hypothalamic-pituitary-adrenal cortex axis, are commonly detected after dehorning. Increases in cortisol concentrations that are evident in animals submitted to a procedure but not in animals given a nerve block or some kind of analgesia, are considered to be indicative of pain (Morton *et al*, 1985; Mellor *et al*, 2005). The same happens with certain behaviours whose incidence is higher during or after a painful procedure. The behaviours recorded in hot-iron disbudding are: backing, falling, bellowing, ear-flick, head-shake, head-rub and quick transitions from standing to lying (Morisse *et al*, 1995; Graf *et al*, 1999; Faulkner *et al*, 2000; Vickers *et al*, 2005; Doherty *et al*, 2007).

In this study we attempted to measure the effect of hot iron disbudding on cortisol and behaviour of calves given xylazine with or without a nerve block. In this way we wanted to assess whether sedation had benefits other than reducing the struggling during the procedure.

MATERIAL AND METHODS

Farm and Animals

The study was carried out in a 1,000 milking cow dairy farm. Forty one female Holstein calves with ages ranging from 29 to 41 days were used in this study. The calves were kept in groups of 10 in a pen floored with wood-shavings. Acid treated milk was permanently

available in a large container with several teats for ad libitum drinking. Hay, calf-starter and water were also available.

The “Centro de Investigação Interdisciplinar em Sanidade Animal” (CIISA) Committee for post-doc studies, of the Lisbon Faculdade de Medicina Veterinaria, approved all animal use in this project. The disbudding protocol was the same as that usually carried out at the farm. Routine use of local anaesthesia in calves submitted to disbudding was introduced as a result of this study.

Procedures

The 41 calves were randomly assigned to one of the following groups: hot-iron disbudded after xylazine (**DX**) n=10; hot-iron disbudded after xylazine and lidocaine (**DXL**) n=11; sham-disbudded control after xylazine and lidocaine (**CXL**) n=10; sham-disbudded after saline and lidocaine (**CL**) n=10.

Xylazine (1ml, aprox. 0.2 mg/kg) or, when applicable, 1ml saline were given intramuscularly 10 min before disbudding. Lidocaine 2% (5ml), without adrenaline, was given s/c, bilateral, on the cornual nerve, mid-way between the base of the horn and the eye lateral cantus just ventral to the frontal bone lateral edge (Nordsy, 1992; Greene, 2003). Blood was collected by jugular venipuncture 5 min after treatments, when all animals that were given xylazine were recumbent and fully sedated. Dehorning was then performed with a butane heated hot-iron, by placing the device over each horn bud for 20 to 30 seconds. Both sham-disbudded groups were submitted to the same procedure but a cold device was applied to each bud for the same time. The procedure was performed always in the same pen, at the same time of day and by the same operator. After disbudding blood was collected, by an experienced veterinarian, at 10, 25, 40 and 60 min by jugular venipuncture.

All behaviour assessment was by the same person, who was blind to treatments. During the procedure each calf reaction was graded by the observer from 0 = no struggling to 5 = severe struggling. Five pain-related behaviours (ear-flicking, head-shaking, head rubbing with hind foot; transitions from lying to standing and back to lying; vocalizations) were then recorded for periods of 5 min just before each blood sampling.

Statistical Analysis

Distributions of the variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used (SPSS 15 for Windows®). Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance.

RESULTS

There was no difference in mean age between groups.

The degree of struggling during disbudding is presented in Fig. 1. The group disbudded after xylazine only, struggled more than sham-disbudded after xylazine ($P < 0.001$) and that sham-disbudded with no sedative ($P = 0.002$). The sham-disbudded with no sedative, CL, showed more struggling than the sham-disbudded after xylazine, CXL, ($P = 0.011$).

Plasma cortisol concentrations are presented in Table 1. All groups given xylazine showed a higher cortisol level at all times compared with animals not sedated ($P < 0.002$). There were no differences in base-line levels among groups of animals sedated with xylazine. For the group disbudded after xylazine but no anaesthesia DX, cortisol concentrations at 10min were higher than at all other times ($P < 0.005$). This group also

showed lower cortisol at 60 min compared with 25 and 40 min. Animals disbudded after xylazine and local anaesthesia DXL showed a reduction in cortisol at 40min ($P = 0.016$) and 60min ($P = 0.026$) compared with 10min. Sham-disbudded with xylazine CXL showed lower cortisol values at 60 min compared with base-line ($P = 0.007$). Sham-disbudded with no sedative calves CL did not show differences across time.

The behaviour analysis is presented in Table 2. At 10min disbudded calves without anaesthesia DX showed more pain-related behaviours than those disbudded after anaesthesia DXL ($P = 0.008$), sham-disbudded after xylazine CX ($P = 0.001$) and sham-disbudded with no sedative CL ($P = 0.004$). At all other times the disbudded with xylazine alone DX calves showed more pain-related behaviours than the non-sedated ones CL ($P < 0.001$), but compared with animals disbudded with xylazine plus anaesthesia DXL and sham-disbudded with xylazine CXL there were only differences at 40min ($P = 0.005$ and 0.023 , respectively). The incidence of pain-related behaviours at 60min was significantly higher in all animals given xylazine (DX, DXL, CXL) compared with non-sedated ones CL ($P < 0.001$). Single behaviour analysis (Table 3) showed that the only behaviour that differed between groups was ear-flicking (at 10min) and ear-flick and transitions (at 60min).

DISCUSSION

The increase in plasma cortisol in the DX group at 10min shows that pain-induced distress caused by disbudding is intense, even in sedated animals. This was to be expected as xylazine does not have an anaesthetic effect (Flecknell, 2000) and so, for surgical procedures in cattle, it should be supplemented with a local anaesthesia (Greene, 2003). However in our study this increase was temporary and showed no difference compared with the other xylazine treated groups. This may be explained by the “ceiling effect” that

occurs when very high levels of cortisol are attained (Mellor, 2005) and because base-line levels of all xylazine-treated animals were already high compared with the non-sedated group. This shows one disadvantage of using plasma cortisol to distinguish severe degrees of pain or when other factors cause a high base-line cortisol level.

The very high cortisol concentrations in all groups given xylazine is an interesting finding. Faulkner *et al* (2000) had already shown that plasma cortisol concentration increases in animals given xylazine even before any procedure is carried out. Alpha-adrenergic agonists reduce the tonic activity of the baroreflex, decreasing arterial pressure and causing bradycardia (Campbell *et al*, 1979; Brest *et al*, 1980) and reduce tissue oxygenation (Hodgson *et al*, 2006). This may be a cause of distress to animals. But it also causes muscle relaxation limiting the ability of the animal to react or avoid human proximity and contact. An increase in eye movements and ear flick, when sedated calves were approached for blood collection (data not shown) suggests that although incapable to react in a more active way, calves are conscious and perhaps fearful of a human presence. Although it is impossible to determine whether a physiological or psychological factor contributes most to this cortisol response, these results suggest that calves heavily sedated and recumbent are in severe distress when approached and handled. This is true even if no painful procedure is performed indicating that it is not a pain-related effect. Stafford *et al* (2003) showed some similar results when studying scoop dehorning. Grondahl-Nielsen *et al* (1999) showed that lidocaine blocking was more effective than xylazine plus butorphanol in lowering serum cortisol suggesting that the former was more efficient in controlling pain. However our findings suggest that high levels of cortisol in xylazine sedated animals are not necessarily related to pain and so should be interpreted with

caution. Nevertheless, we also found that local anaesthesia reduces the cortisol increase that is seen at 10 min in non-blocked disbudded calves.

At 60 min cortisol levels were lower than base-line in all xylazine treated groups, although only significant in the sham-disbudded animals. This was to be expected as xylazine has a short half-life in cattle (36min) and behaviour analysis showed that physical activity started to increase 40 min after xylazine injection. The more rapid decrease of cortisol values in the sham-disbudded animals may be due to the absence of pain-induced distress in this group.

Mish *et al* (2008) state that although sedated calves will not respond to the dehorning procedure they do feel it because xylazine does not possess any anaesthetic activity. In our study we showed that calves treated only with xylazine do respond to the thermo-cautery and that pain can be assessed by the degree of struggling during disbudding. The lower response of disbudded calves that were blocked demonstrates that xylazine alone is not sufficient to control pain during the procedure. The pain-related signs are not those shown in other studies with calves not sedated (backing, lifting front legs etc...) but are evident to an experienced observer and should not be underestimated. In contrast, there were no differences in the degree of struggling between sham-disbudded animals and those disbudded after lidocaine nerve block. Although some authors (Petrie *et al*, 1996; Vickers *et al*, 2005) did not find lidocaine to have a significant effect on pain control, others did show a benefit (Graf *et al*, 1999; Grondahl-Nielsen *et al*, 1999; Faulkner *et al*, 2002; Doherty *et al*, 2007). The difference may be due to the anaesthesia technique being that s/c injection of 5ml of lidocaine mid-way between the horn base and the eye seems to give better results than injections of 3 ml (Petrie *et al*, 1996) or the injection at the base of the horn (Vickers *et al*, 2005).

Faulkner *et al* (2000) looked at the effect of disbudding on the behaviour of calves given xylazine but only studied the effect from three to 24 hours after the procedure. In our study we looked at behaviours for the first hour after disbudding to try to identify which pain-related behaviour should be used in evaluating early pain in calves submitted to hot-iron dehorning after xylazine injection. Very soon after hot-iron disbudding the pain in animals without a block is sufficiently intense to cause a difference in the incidence of behaviours and is also associated with an increase in plasma cortisol concentration. At this time ear-flick is the only behaviour to differ between groups and so should be considered essential to assess pain in xylazine-sedated animals

The characteristic vocalization associated with xylazine sedation was noted in all groups given xylazine and so should not be used as a sign of pain in animals treated with α 2-agonists. The same happens with transitions that occur in all sedated animals when xylazine effect is beginning to subside (over 45 min after injection).

In conclusion we suggest that, although restraining and disbudding is certainly much easier when calves are sedated and recumbent, calves given xylazine are exposed to severe distress even if no painful procedure is carried out. There was no effect of treatment on many of the behavioural measures because the sedation inhibits active movements during the first 30 min and because of the type of behaviours shown by cattle when recovering from the effect of xylazine. Only struggling during the procedure and ear flicking immediately after the disbudding, are useful indicators of the degree of pain caused by hot-iron cauterisation in xylazine-sedated calves.

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Table 1. Plasma cortisol concentration (mean \pm SD) of calves disbudded with hot-iron after xylazine sedation

Group	n	Time from disbudding				
		-5 min	+10 min	+ 25 min	+ 40 min	+ 60 min
CL	10	16.85 \pm 10.19 ^{aA}	18.54 \pm 11.37 ^{aA}	16.17 \pm 6.90 ^{aA}	10.85 \pm 3.67 ^{aA}	10.19 \pm 4.62 ^{aA}
CXL	10	60.76 \pm 24.18 ^{bAB}	77.78 \pm 33.29 ^{bA}	68.29 \pm 48.77 ^{bAB}	51.45 \pm 37.63 ^{bBC}	33.20 \pm 28.06 ^{bC}
DX	10	53.00 \pm 24.79 ^{bACD}	94.82 \pm 19.53 ^{bB}	76.89 \pm 21.19 ^{bA}	54.22 \pm 23.75 ^{bC}	37.17 \pm 21.58 ^{bD}
DXL	11	64.12 \pm 35.94 ^{bAB}	96.58 \pm 40.04 ^{bA}	86.43 \pm 59.45 ^{bAB}	70.94 \pm 43.87 ^{bB}	59.61 \pm 36.55 ^{bB}

CL: calves sham-disbudded after treatment with regional lidocaine; CXL: calves sham-disbudded after treatment with xylazine and regional lidocaine; DX: calves disbudded after xylazine;DXL: calves disbudded after xylazine and regional lidocaine.

Different lower case superscript letters indicate difference between groups.

Different upper case superscript letters indicate difference across time.

Table 2. Mean \pm SD incidence of pain-related behaviours of calves disbudded with hot-iron

Group	n	Time from disbudding			
		+10 min	+ 25 min	+ 40 min	+ 60 min
CL	10	0.50 \pm 0.71 ^b	0.4 \pm 0.70 ^b	0.50 \pm 0.71 ^b	0.20 \pm 0.63 ^b
CXL	10	0.20 \pm 0.42 ^b	0.60 \pm 1.35 ^a	1.20 \pm 1.75 ^b	2.50 \pm 2.07 ^a
DX	10	2.40 \pm 1.71 ^a	1.90 \pm 1.60 ^a	3.20 \pm 1.55 ^a	2.80 \pm 1.55 ^a
DXL	11	0.64 \pm 0.81 ^b	0.55 \pm 0.93 ^{ab}	1.00 \pm 1.67 ^b	2.18 \pm 0.87 ^a

CL: calves sham-disbudded after treatment with regional lidocaine; CXL: calves sham-disbudded after treatment with xylazine and regional lidocaine; DX: calves disbudded after xylazine; DXL: calves disbudded after xylazine and regional lidocaine.

Different lower case superscript letters indicate difference between groups.

FIG.1 (caption)

Fig.1. Degree of struggling (mean \pm SD) during hot-iron disbudding (scale from 0 = no struggling to 5 = severe struggling). CL (n=10) – sham-disbudding after cornual nerve blocking with lidocaine; CXL (n=10) – sham-disbudding after i/m xylazine and cornual nerve blocking with lidocaine; DX (n=10) – disbudding after i/m xylazine; DXL (n=11) – disbudding after i/m xylazine and cornual nerve blocking with lidocaine.

Different superscript letter indicates differences between groups.

STRUGGLING AT DISBUDDING

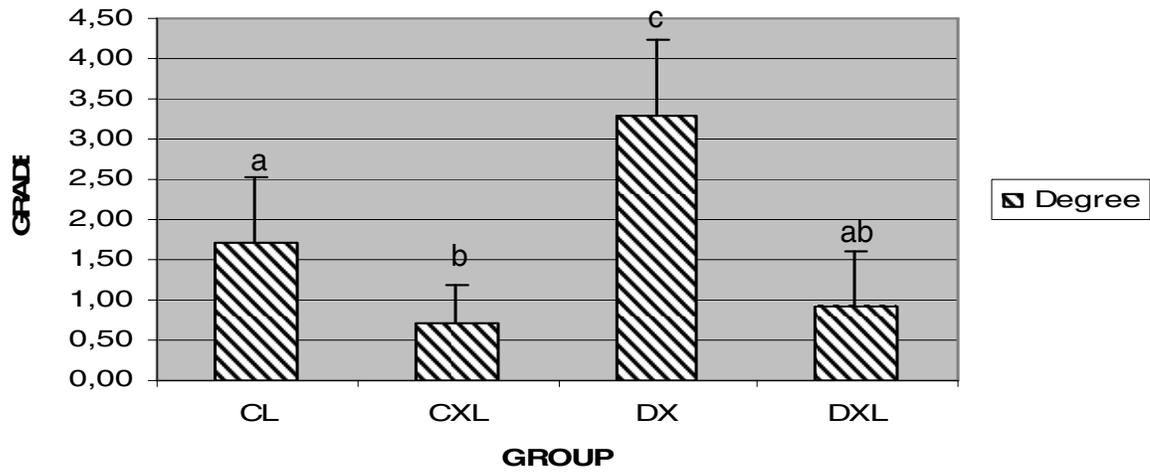


Table 3. Incidence of pain-related behaviours (mean \pm SD) of calves for the first hour post-disbudding with hot-iron

Time	Group	Incidence of all behaviours	Specific Behaviour				
			Ear flick	Head shake	Head rub	Transitions	Vocalization
+10 min	CL	0.50	0.20 \pm 0.42a	0.20 \pm 0.42a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
	CXL	0.20 \pm 0.42a	0.20 \pm 0.42a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
	DX	2.40 \pm 1.71b	2.00	0.20 \pm 0.42a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.20 \pm 0.42a
	DXL	0.64 \pm 0.81a	0.45 \pm 0.52a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.18 \pm 0.40a
+25 min	CL	0.40 \pm 0.7a	0.10 \pm 0.32a	0.20 \pm 0.42a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.10 \pm 0.32a
	CXL	0.60 \pm 1.35b	0.20 \pm 0.42ab	0.10 \pm 0.32a	0.00 \pm 0.00a	0.10 \pm 0.32a	0.20 \pm 0.63a
	DX	1.90 \pm 1.60b	1.10 \pm 1.10b	0.40 \pm 0.70a	0.30 \pm 0.67a	0.00 \pm 0.00a	0.10 \pm 0.32a
	DXL	0.55 \pm 0.93ab	0.27 \pm 0.65ab	0.09 \pm 0.30a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.18 \pm 0.40a
+40 min	CL	0.5 \pm 0.71a	0.20 \pm 0.42a	0.10 \pm 0.32a	0.00 \pm 0.00a	0.10 \pm 0.32a	0.10 \pm 0.32a
	CXL	1.20 \pm 1.75a	0.60 \pm 1.07 ^a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.40 \pm 0.52a	0.20 \pm 0.63a
	DX	3.20 \pm 1.55b	1.00 \pm 0.94 ^a	0.60 \pm 0.70a	0.30 \pm 0.67a	0.70 \pm 0.82a	0.60 \pm 0.70a
	DXL	1.00 \pm 1.67a	0.18 \pm 0.60a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.36 \pm 0.67a	0.45 \pm 0.69a
+60 min	CL	0.20 \pm 0.63a	0.10 \pm 0.32a	0.10 \pm 0.32a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
	CXL	2.50 \pm 2.07b	1.20 \pm 0.92b	0.40 \pm 0.84a	0.00 \pm 0.00a	0.70 \pm 1.06b	0.20 \pm 0.42a
	DX	2.80 \pm 1.55b	1.70 \pm 1.06b	0.10 \pm 0.32a	0.40 \pm 0.70a	0.40 \pm 0.70b	0.20 \pm 0.42a
	DXL	2.18 \pm 0.87b	1.50 \pm 0.53b	0.00 \pm 0.00a	0.00 \pm 0.00a	0.45 \pm 0.52b	0.36 \pm 0.50a

CL (n=10) sham-disbudding after cornual nerve blocking with lidocaine; CXL (n=10) sham-disbudding after i/m xylazine and cornual nerve blocking with lidocaine; DX (n=10) disbudding after i/m xylazine;DXL (n=11) disbudding after i/m xylazine and cornual nerve blocking with lidocaine.

Different superscript letters in each period of time indicate difference between groups for which $P < 0.001$