Reprod Dom Anim **45**, 160–167 (2010); doi: 10.1111/j.1439-0531.2008.01295.x
ISSN 0936-6768

Assessment of Differences in Some Indicators of Pain in Double Muscled Belgian Blue Cows Following Naturally Calving vs Caesarean Section

I Kolkman[1,2], S Aerts[3], H Vervaecke[1], J Vicca[1], J Vandelook[1], A de Kruif[2], G Opsomer[2] and D Lips[1,3]

[1]*Ethology & Animal Welfare group, Department of Agro- and Biotechnology, KaHo Sint-Lieven/Association University of Leuven, Sint Niklaas, Belgium;* [2]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;* [3]*Centre for Science, Technology and Ethics, University of Leuven, Leuven, Belgium*

Contents

This article describes a study of the behaviour of double muscled Belgian Blue (BB) cows during the *peri partum* period to assess the differences in pain perception in cows calving *per vaginam* vs cows delivering by caesarean section (CS). In one herd, a total of 30 multiparous cows, of which 17 delivered by CS and 13 calved *per vaginam*, were closely observed at approximately 1 month before calving and at days 1, 3 and 14 after parturition. The main behavioural indicators of pain were alertness, transition in posture from standing to lying and vice versa, aggressive behaviour, vocalization, rumination quality, reaction on wound and vulva pressure and the percentage of visible eye-white. The main significant differences were lower overall activity and more transition in posture in animals that delivered by CS than in cows that calved naturally. Less time was spent on eating and ruminating in the CS group, their total resting time was longer and their total standing time was shorter. These significant differences were only observed on the first day after calving. Cows of the CS group reacted significantly more when pressure was put on the left flank on the first, third and fourteenth day after calving, whereas animals that calved *per vaginam* showed more reaction when pressure was put on the area around the vulva, but only on the first day. Based on the results of the present study, we can conclude that there are some significant short-term behavioural differences between BB cows that calve naturally and those that deliver by CS, but in general, the differences are subtle and of short duration.

Introduction

The double muscled Belgian Blue (BB) cattle breed is valued for its extreme muscularity and superior carcass and meat quality. Unfortunately, it is also criticized for its calving difficulties (Lips et al. 2001). In Belgium, where the cost of a caesarean section (CS) is very low and the price of the calf relatively high, where veterinary practitioners can easily be reached and are very experienced in performing CS, 95–99.9% of the double muscled BB cows are delivered by CS (Hanset 2002; Kolkman et al. 2007). In many cases, practising veterinarians carry out a very basic gynaecological examination when they are confronted with a BB cow in labour and start to perform a CS immediately. Therefore, the CS in double muscled cows is often referred to as an elective CS (Kolkman et al. 2007). This is currently experienced as an example of animal instrumentalisation, which, in some countries, causes an aversion towards this breed. This complaint is based on the general belief that delivery per CS causes more pain and discomfort than delivery *per vaginam*. Two questions arise: (i) does a correctly performed CS under local anaesthesia (Kolkman et al. 2007) cause significantly more pain and distress than natural delivery? (ii) which proportion of cows experience chronic pain as a consequence of post-operative complications? (Webster 2002). Although veterinarians in Belgium are very well experienced in performing a CS, it remains a major abdominal operation performed in a contaminated environment and hence, it cannot be considered a sterile surgical procedure. As the success rate of CS and thus its impact on animal welfare mainly depends on the operation technique, it is imperative to perform the surgical procedure according to recognised best practice (Mijten 1994; Kolkman et al. 2007).

An objective assessment of pain and discomfort in animals is known to be very difficult (Bourne et al. 2005). Pain, stress and distress produce similar behavioural, biochemical and physiological adaptations, finally leading to the well known 'fight or flight' reaction, which is based on a stimulation of the hypothalamic-pituitary-adrenal (HPA) axis (Bourne et al. 2005). Practical experience has shown that pain in cattle is usually linked to typical clinical signs and behavioural changes such as a significant decrease in food intake and grooming. Ruminants in significant pain often appear dull and depressed, hold their heads low, and show little interest in their surroundings (Phillips 2002; Bourne et al. 2005). Common alterations in physiology that indicate pain can be monitored, such as heart rate (Lay et al. 1992), respiratory rate, body temperature (Mellor and Stafford 1999), increase in blood pressure (Jourdan et al. 2001) and changes in digestive system (bodyweight loss, variation in faeces volume) or locomotory system (tremors, hyperaesthesia; Morton and Griffiths 1985). In response to a stimulus, animals can show modifications in attitude (immobility) or in motor activity (jumping, withdrawal of a limb; Jourdan et al. 2001). Severe pain often results in a rapid, shallow respiration and grunting or grinding of teeth might be heard (Flecknell and Waterman-Pearson 2000). Therefore, pain can be assessed by observing these physiological responses while monitoring the animals' behaviour (Molony et al. 1995; Molony and Kent 1997; Grandin 1998, 2001; Sandem et al. 2002, 2005). Molony et al. (1995) used restlessness, foot stamping/kicking, tail wagging, easing quarters and head turning to assess acute pain after different methods of castration of calves. Postural changes or changes in locomotor activity like rolling, jumping, easing quarters, licking and biting at the side of damage were assessed by

Molony and Kent (1997) in their study of the responses of lambs and calves to castration and of lambs to taildocking.

In general, studies on behavioural responses to pain have their limits, as behavioural responses to pain are difficult to measure and show marked differences between different species. These differences reflect the unique behavioural repertoire of the species (Sanford et al. 1986). Nevertheless, responses of related species to similar stimuli can also differ. For instance, lambs and calves castrated with rubber rings exhibit different behaviours (Mellor et al. 1991; Mellor and Stafford 1999) and experts found CS to be more painful in cows than in ewes (Fitzpatrick et al. 2002). An animal's age and its experience may also influence behavioural responses to particular procedures. Hormone-related differences have been well documented, as have the effects of late pregnancy and parturition. During oestrus, late pregnancy and immediately after parturition, the nociceptive threshold shows an apparent increase (Cook 1997, 1998). On the other hand, assessing behavioural responses is non-invasive and thus not harmful for the animals in contrast to invasive methods such as measuring plasma cortisol levels. Furthermore, the blood sampling itself causes stress which may significantly affect the results (Queyras and Carosi 2004).

To assess pain and discomfort in cattle following surgery, the most indicative signs to monitor were stated to be anorexia, vocalisation and grinding of the teeth (Watts 2001). The heart rate, respiratory rate, blood cortisol levels and (withdrawal) reaction following palpation of the wound are also known to be reliable indicators of post-operative pain in recently operated cattle (Watts 2001).

The aim of this study was to examine differences in pain and discomfort in double muscled BB cows after giving birth by vaginal delivery vs CS.

Materials and Methods

Animals and management

The study was conducted from December 2005 until March 2006 on a BB herd with an uncommonly large fraction of cows giving birth *per vaginam*. Thirty multiparous cows were included in the study, 13 of which calved *per vaginam* while the other 17 were delivered by CS. All animals included in the study were characterized by an extreme muscularity (double muscled, S carcass animals; SEUROP carcass classification system (European Community 2003) and were kept in tie-stalls, tethered by neck chains. Calves were housed in small boxes in the tie-stall behind the cows. One compartment of the tie-stall contained four calves and four cows, allowing the calves to suckle all four cows. All animals were well acclimatized to the shed before observation took place. The feeding regimen, consisting of corn silage and concentrates in the morning and hay in the evening, was identical for all participating cows. Yet, cows that were delivered by CS were not fed during the first 24 h following surgery to prevent coalescence of the rumen and peritoneum.

At the moment of parturition, the veterinarian decided in accord with the farmer whether a CS was necessary. Before each delivery, the cow was laid down and a trial extraction was performed to check the possibility of natural calving using the force of one man. The trial extraction was positive if the metacarpal joints could be pulled out for at least one hand's breadth and when the nose of the calf was clearly visible. In case of a positive trial extraction, the calf was pulled out by the farmer, by the farmer and the vet or with the help of a calf aid at the cow's place in the tie-stall. Caesarean sections were performed in a specifically designed CS box by two different veterinarians according to the surgical technique described by Kolkman et al. (2007). The procedure was performed under local anesthesia on the left flank of a standing cow. No post-operative pain relief was administered. The calf was immediately brought to a clean box behind the mother in the tie-stall. First, the farmer helped the calf to suckle its own mother while after that all four calves were allowed to suckle the four cows in the compartment *ad libitum*.

Complications after both delivery types (e.g. tearing after extraction, retained placenta, and wound infection) were treated according to recognised best practices by the veterinarians.

Observations

One month before the expected day of calving (D-30), and on the first (D1), third (D3) and 14th (D14) day after calving, the animals were closely observed. Observations were conducted three times a day: 1 h after each meal (i.e. 9 AM and 8 PM) and at 2 PM, each session lasting 45 min. The time point of first observation depended on the moment of calving: when parturition occurred during the late evening or night, the observation started the next morning at 9 AM; when the calving took place during the morning, the observation started at 2 PM and when the partus occurred during the afternoon, the observation started at 8 PM.

To give the animals the opportunity to acclimatize to the presence of the observer, observations started half an hour after the observer assumed his position in the shed. Observations were carried out by five different observers using the OBSERVER® 5.0 software package (Noldus Ltd, 2003, Wageningen, The Netherlands). The five observers were trained intensively on how to use this software package and to make them familiar with the definitions used (Table 1) and the procedures applied (e.g. pressure testing at the CS wound and the vulva) to guarantee standardization of the results. The inter-observer reliability was checked through a t-test and the observers were balanced equally between the cows that were delivered *per vaginam* and by CS.

The inclusion of two different veterinarians that performed the CS and five different observers may have had an influence on the outcome of the results. To minimize the bias caused by the veterinarians, they were carefully instructed to use the same criteria when performing the trial extraction and to carry out the CS (Kolkman et al. 2007). It was an ergonomic choice to incorporate five observers instead of one, but variations between their observations were reduced by carefully training them before the experiment started. Bias caused by including several veterinarians and observers was

Table 1. Descriptions of behaviour recorded during the experiment

Behaviour	Description
Independent variables	
Number of animals	Ear number of the animal
Observer	The person who observed
Presence of food	Concentrate (C): full (F), half full (HF), or nothing (N); hay (H): full, half full or nothing
Day	*pre partum* day (D-30), day 1 (D1), 3 (D3) or 14 (D14) *post partum*
Number of calving/CS	The number of CS or natural calving the cow already experienced before
Moment of observation (MO	Morning (M), afternoon (A), evening (E)
The present calving	Caesarean section (CS), naturally calving (N) or not relevant (NR; *pre partum* period)
Reaction on pressure	Reaction after firm pressure on the left flank, the right flank (control) or the vulva (performed after the observation)
General activity	
Overall activity	Index of general activities
Limb movement	Movements of front or hind limbs
Ear flicking	Vigorous movement of one or both ears; independent of shaking of the head
Nose licking	Cleaning of the muzzle
Licking itself	Licking its own body except wound
Look at/sniff at neighbour	Looking or sniffing at its neighbour
Pain indicators	
Alertness	
Reactivity on noise	The observer makes a loud noise i.e. played a mobile telephone tune before the observation starts and scores the reaction of the cow
Mean alertness	Overall score of the mean impression of alertness
Transition in posture	Number of times a cow stood up and laid down; each unit scored included both the act of rising and lying down
Aggressive behaviour	Number of times a cow behaved aggressively against her environment, calf or neighbour
Vocalisation	Occurrence of each vocal sound was recorded; this includes sound made towards her calf, loud and soft
Lip curl	Curling of the upper or both lips, including flehmen; flehmen is defined as curling of the upper lip combined with head elevation
Rumination quality	The mean number of chewing per bolus
Flank pressure	Giving pressure on the left flank (operation side) and on the right flank (control); difference is made between no reaction (0) and reaction after firm pressure (1)
Vulva pressure	Giving firm pressure on the vulva on both sides; difference is made between No reaction and reaction after firm pressure
Eye white	Assessed by evaluation of the overall eye white during the 45 min; no eye white, eye white seen once or twice; eye white seen more than twice
Activity budget	
Eating	Total time spent eating
Rumination	Total time spent ruminating
Lying (left or right)	total time spent in ventral recumbency with the legs tucked in and the head up or down, either to one side or directly in front
Standing	Total time spent standing
Leaning	Time spent resting with the nose on the border of the groove of the chain that is used

furthermore limited as both 'veterinarian' and 'observer' were included as a fixed factor in the statistical model.

Statistical analysis

All statistical analyses were performed using R software (version 2.6) for the explorative analysis and the SAS procedures MIXED, GMIMMIX or NLMIXED (version 9; S.A.S. Institute, Tervuren, Belgium) for the mixed models. For the statistical assessments, all behavioural indicators were summed per day to compensate for the circadian pattern. The CS and naturally calving animals were both clustered to minimize the effect of individual variations between animals. According to Molony and Kent (1997), clustering behavioural scores has statistical advantages over using individual behaviours. On the other hand, there is always a danger of exaggerating effects if the incidences of dependent behaviours are summed (Molony et al. 1995). Therefore, 'general activity' should not be used as the sole pain or welfare indicator.

Explorative statistical analyses were performed by a non-parametric Wilcoxon-test and a Fisher exact test on all four observational days to compare animals that calved by CS with animals that calved by vaginal delivery. To take the clustered nature of the data (4 times measurements per animal) into account, statistical analysis was carried out based on using a mixed model including a random effect for animal. The fixed effects of these models are treatment and the day of observations. The response is one of variables which describe the activity of the animal (reaction on noise, eye white, wound and vulva pressure). Both the veterinarian and the observer were taken into account in the mixed model. The animals were divided into two treatment groups: delivery by CS vs calving *per vaginam*. Using this model, it was possible to compare the time evolution between the two treatment groups as well as to look within each group for significant differences in the activity of the cows between the *post partum* observation days (D1, D3, D14) and the *pre partum* observation day (D-30).

Results

General results

Almost 60% of the CS animals were of second parity (58.8%) while of the natural calving group, a majority had calved five times or more (61.5%) (Data not shown). No wound infections were noticed after CS. Three cows retained the placenta (two after CS and one after natural calving) while one animal suffered from a vaginal laceration after extraction.

A total of 30 calves were born of which six died within the first 48 h after parturition. These deaths were not linked to the parturition but to a diarrhoea epidemic. In Table 2, the gender of the calves is summarized per type of calving.

Comparison between the naturally calving and the CS group

Before calving (D-30), there was no significant difference in general activity, the activity budget, and in the relief of pain measured by using pain indicators between the naturally calving animals and the ones that were delivered by CS. The overall results of the time evolution only show significant differences in reaction on wound pressure for the CS group ($p < 0.01$) and pressure on the vulva for the naturally calving group ($p < 0.01$). Animals that were delivered by CS had significantly more reaction during the whole observation period when pressure was put on their left flank compared with animals that delivered *per vaginam*, whereas the latter animals showed more reaction when the area around the vulva was touched.

Day one post partum

After a CS, animals had significantly ($p < 0.05$) less limb movements and had more transitions in posture ($p < 0.001$) in comparison with cows that calved *per vaginam* (Table 3). In the CS group, the rumination quality was lower and less time was spent eating ($p < 0.001$; Table 3). Results also demonstrated a difference in total resting and standing time ($p < 0.001$), the resting time being longer and the standing time shorter within the CS group. When lying down, CS animals laid more on their right site ($p < 0.001$; Table 3). Finally, cows of the CS group reacted significantly more when pressure was put on their left flank, whereas animals that calved naturally showed more reaction when the area around the vulva was touched ($p < 0.05$; Table 3).

Day three post partum

There was only a significant difference in the time spent eating ($p < 0.05$) and the reaction of the animal to wound pressure ($p < 0.05$). Animals delivered by CS spent more time eating ($p < 0.05$) and reacted more upon pressure on the left flank (Table 4).

Table 2. Gender of the calves born by CS or *per vaginam*

		Amount	
		Alive	Dead[1]
Caesarean Section	Male	6	2
	Female	8	1
Calving *per vaginam*	Male	6	1
	Female	4	2

[1]Deaths within 48 h, these deaths were not linked to parturition but to a diarrhoea epidemic.

Table 3. Comparison of general activity, pain indicators and activity budget between the naturally calving and CS group on the first day after calving (D1; 3 × 45 min observation)

Observations	CS n = 17	Natural calving n = 13	Probability (Mixed Model or χ^2)
General activity (count)			
Overall activity	251 ± 134	388 ± 214	p = 0.052
Limb movements	214 ± 126	349 ± 192	p = 0.042*
Ear flicking	2.9 ± 4.0	1.7 ± 2.2	p = 0.694
Nose licking	9.4 ± 6.5	13.2 ± 16.5	p = 0.323
Licking itself	4.5 ± 5.0	5.3 ± 4.9	p = 0.751
Look at/sniff at neighbour	20.5 ± 15.4	18.2 ± 16.0	p = 0.807
Pain indicators (count)			
Transition in posture	5.5 ± 2.0	3.7 ± 0.9	p < 0.001**
Aggressive behaviour	0.7 ± 1.7	0.9 ± 2.6	p = 0.814
Vocalisation (loud and soft)	24.6 ± 31.3	49.2 ± 91.0	p = 0.335
Vocalisation loud	3.2 ± 5.1	3.2 ± 5.1	p = 0.095
Vocalisation soft	20.3 ± 28.6	15.7 ± 16.2	p = 0.716
Lip curl	0.59 ± 1.50	0.38 ± 0.65	p = 0.738
Rumination quality	47 ± 18	66 ± 13	p < 0.001**
Reaction to noise			
Reaction	29%	31%	p = 0.935
No reaction	71%	69%	
Wound pressure left			
Reaction	94%	31%	p = 0.016*
No reaction	6%	69%	
Wound pressure right			
Reaction	6%	15%	p = 0.412
No reaction	94%	85%	
Vulva pressure			
Reaction	12%	62%	p = 0.014*
No reaction	88%	38%	
Eye white			
No eye white	12%	8%	p = 0.485
Eye white seen once or twice	41%	31%	
Eye white seen more than twice	47%	62%	
Activity budget (in s)			
Eating	595 ± 602	1998 ± 775	p < 0.001**
Rumination	1680 ± 1264	2471 ± 1578	p = 0.101
Lying (left or right)	4802 ± 1948	2372 ± 2472	p < 0.001**
Lying left	1421 ± 1755	1174 ± 1993	p = 0.665
Lying right	3382 ± 2209	1198 ± 1994	p < 0.001**
Standing	3292 ± 1945	5728 ± 2472	p < 0.001**
Leaning	107 ± 277	78 ± 154	p = 0.751

Values represent the mean ± 1 SD.
*p < 0.05, **p < 0.001.

Day fourteen post partum

Animals in the CS group did not only show a more sensitive reaction after pressure on the left flank, but also showed more interest in their neighbour by sniffing ($p < 0.05$). Vocalisation, both loud and soft, occurred more frequently in the naturally calving group ($p < 0.05$; Table 5).

Calving *per vaginam* group

General activity

No significant differences in general activity were seen on D1, D3 and D14 compared to observations made before parturition (D-30; Table 6).

Table 4. Comparison of general activity, pain indicators and activity budget between the naturally calving and CS groups on the third day after calving (D3; 3 × 45 min observation)

Observations	CS n = 17	Natural calving n = 13	Probability (Mixed model or χ^2)
General activity (count)			
Overall activity	294 ± 94	377 ± 155	p = 0.233
Limb movements	231 ± 78	326 ± 142	p = 0.149
Ear flicking	6.8 ± 18.4	5.8 ± 9.2	p = 0.761
Nose licking	11.2 ± 6.6	12.0 ± 9.5	p = 0.831
Licking itself	9.7 ± 5.4	7.3 ± 8.9	p = 0.330
Look at/sniff at neighbour	35.8 ± 25.2	25.8 ± 28.8	p = 0.290
Pain indicators (count)			
Transition in posture	4.8 ± 1.6	4.4 ± 0.9	p = 0.477
Aggressive behaviour	0.4 ± 1.3	1.1 ± 2.2	p = 0.471
Vocalisation (loud and soft)	31.0 ± 26.0	25.0 ± 25.0	p = 0.816
Vocalisation loud	5.9 ± 10.6	3.5 ± 5.9	p = 0.653
Vocalisation soft	11.1 ± 14.9	14.9 ± 18.9	p = 0.763
Lip curl	1.41 ± 3.710	0.23 ± 0.44	p = 0.055
Rumination quality	64.9 ± 8.9	67.5 ± 9.4	p = 0.83
Reactivity on noise			
Cow reacts	24%	23%	p = 0.979
Cow does not react	76%	77%	
Wound pressure left			
Cow reacts	82%	31%	p = **0.029***
Cow does not react	18%	69%	
Wound pressure right			
Cow reacts	18%	31%	p = 0.414
Cow does not react	82%	69%	
Vulva pressure			
Cow reacts	6%	31%	p = 0.116
cow does not react	94%	69%	
Eye white			
No eye white	24%	23%	p = 0.533
Eye white seen once or twice	41%	23%	
Eye white seen more than twice	35%	54%	
Activity budget (in s)			
Eating	2103 ± 960	1171 ± 719	p = **0.012***
Rumination	3531 ± 1303	3438 ± 1465	p = 0.847
Lying (left or right)	2865 ± 1792	2336 ± 2266	p = 0.457
Lying left	1148 ± 1552	1025 ± 1734	p = 0.829
Lying right	1718 ± 1658	1311 ± 1935	p = 0.527
Standing	5235 ± 1791	5764 ± 2266	p = 0.463
Leaning	21.8 ± 78.2	190.7 ± 418.5	p = 0.058

Values represent the mean ± 1 SD.
*p < 0.05.

Table 5. Comparison of general activity, pain indicators and activity budget between the naturally calving and CS groups on the fourteenth day after calving (D14; 3 × 45 min observation)

Observations	CS n = 17	Natural calving n = 13	Probability (Mixed Model or χ^2)
General activity (count)			
Overall activity	305 ± 181	372 ± 113	p = 0.463
Limb movements	242 ± 159	332 ± 116	p = 0.300
Ear flicking	1.2 ± 1.5	0.6 ± 0.8	p = 0.834
Nose licking	10.3 ± 5.9	15.3 ± 11.5	p = 0.216
Licking itself	9.3 ± 8.2	6.5 ± 5.5	p = 0.386
Look at/sniff at neighbour	41.6 ± 33.6	17.7 ± 33.1	p = 0.029*
Pain indicators (count)			
Transition in posture	4.5 ± 1.1	5.2 ± 1.9	p = 0.224
Aggressive behaviour	1.4 ± 2.8	1.8 ± 2.2	p = 0.661
Vocalisation (loud and soft)	16.8 ± 24.6	80.9 ± 199.6	p = 0.024*
Vocalisation loud	0.9 ± 1.9	12.60 ± 34.65	p = 0.037*
Vocalisation soft	11.4 ± 19.8	45.3 ± 102.2	p = 0.020*
Lip curl	0.38 ± 0.65	0.70 ± 1.06	p = 0.568
Rumination quality	58 ± 13	65 ± 11	p = 0.443
Reactivity on noise			
Cow reacts	31%	40%	p = 0.647
Cow does not react	69%	60%	
Wound pressure left			
Cow reacts	85%	30%	p = 0.033*
Cow does not react	15%	70%	
Wound pressure right			
Cow reacts	23%	30%	p = 0.722
Cow does not react	77%	70%	
Vulva pressure			
Cow reacts	15%	30%	p = 0.420
Cow does not react	85%	70%	
Eye white			
No eye white	23%	40%	p = 0.591
Eye white seen once or twice	31%	20%	
Eye white seen more than twice	46%	40%	
Activity budget (in s)			
Eating	2356 ± 1313	1923 ± 1114	p = 0.200
Rumination	2339 ± 1501	1927 ± 992	p = 0.438
Lying (left or right)	2743 ± 1473	2412 ± 1570	p = 0.725
Lying left	519 ± 874	1267 ± 1461	p = 0.177
Lying right	2224 ± 1437	1145 ± 1183	p = 0.140
Standing	5357 ± 1473	5688 ± 1570	p = 0.732
Leaning	16.7 ± 60.4	182.5 ± 251.7	p = 0.171

Values represent the mean ± 1 SD.
*p < 0.05.

Pain indicators

On D1 and D3 after calving less reaction to noise was noticed in comparison with D-30 ($p < 0.05$). The number of times an animal stood up and laid down was higher on D3 ($p < 0.05$) and D14 ($p < 0.001$) after parturition than before calving (Table 6). On D1 and D3, the cows behaved less aggressively compared with D-30 ($p < 0.05$). Vocalisations had significantly increased on D1 and D14 compared to D-30 ($p < 0.05$; Table 6).

Activity budget

The time spent ruminating, was shorter on D14 than before calving ($p < 0.05$; Table 6).

Caesarean section group

General activity

Results from the mixed models show a significant difference in overall activity on D1 ($p < 0.01$) and D3 ($p < 0.05$) in contrast with observations 1 month before calving (Table 6). As for the general activity, first of all the limb activity was lower on all three days *post partum* compared with that on D-30 ($p < 0.05$). Secondly, the animal was licking itself (including its nose) less often on D1 and flapping its ears more often on D3 in comparison with D-30 ($p < 0.05$; Table 6).

Pain indicators

Table 6 reveals more transitions in posture on D1 than on D-30 ($p < 0.001$). The rumination quality had significantly decreased on D1 and D14 compared to D-30 ($p < 0.001$). Observations on D1 ($p < 0.01$), D3 ($p < 0.001$) and D14 ($p < 0.05$) mention less reaction to noise than on D-30 (Table 6).

Activity budget

The activity pattern of the animals shows some significant differences of the resting, ruminating, standing and

Table 6. Comparison of general activity, pain indicators and activity budget before (D-30) and after (D1, D3, D14) parturition within the CS- and the *vias naturales* groups (3 × 45 min observation)

Observations	D-30-D1		D-30-D3		D-30-D14	
Vias Naterales group	change	p-value	change	p-value	change	p-value
General activity (count)						
Overall activity (instances)	−41	0.566	−51	0.473	−61	0.427
Limb movements	−24	0.714	−47	0.479	−49	0.500
Ear flicking	1.2	0.670	5.3	0.064	0.7	0.824
Nose licking	−4.3	0.244	−5.5	0.136	−2.0	0.621
Licking itself	−1.0	0.631	1.0	0.657	1.0	0.658
Look at/sniff at neighbour	−12.4	0.221	−4.8	0.638	−12.8	0.237
Pain indicators (count)						
Reaction to noise	−2.1	0.033*	−2.5	0.016*	−1.6	0.092
Transition in posture	0.6	0.308	1.3	0.027*	2.1	< 0.001***
Aggressive behaviour	−2.1	0.018*	−1.9	0.027*	−1.2	0.191
Vocalisation (loud and soft)	49	0.062	24	0.348	83	0.004**
Vocalisation loud	12	0.024*	4	0.507	14	0.019*
Vocalisation soft	15	0.244	14	0.268	45	0.002**
Lip curl	0.4	0.471	0.2	0.664	0.7	0.254
Rumination quality	−2.5	0.498	−1.3	0.715	−5.5	0.164
Eye white	−0.3	0.702	0.4	0.645	1.1	0.194
Activity budget (in s)						
Eating	433	0.235	−395	0.278	317	0.420
Rumination	−772	0.117	195	0.690	−1347	0.012*
Lying (left or right)	−743	0.292	−780	0.269	−721	0.344
Lying left	−65	0.902	−214	0.686	82	0.886
Lying right	−679	0.311	−566	0.398	−754	0.297
Standing	743	0.299	780	0.276	721	0.351
Caesarean group	change	p-value	change	p-value	change	p-value
General activity (count)						
Overall activity	−187	0.004**	−144	0.024*	−129	0.059
Limb movements	−168	0.005**	−151	0.011*	−134	0.035*
Ear flicking	1.5	0.542	5.4	0.032*	0.6	0.837
Nose licking	−7.4	0.024*	−5.6	0.860	−6.6	0.061
Licking itself	−4.0	0.036*	11	0.544	1.2	0.555
Look at/sniff at neighbour	−9.1	0.304	6.3	0.477	12.1	0.205
Pain indicators (count)						
Reaction to noise	−2.5	0.008**	−2.8	0.005**	−2.4	0.015*
Transition in posture	1.7	< 0.001***	1.0	0.051	0.7	0.213
Aggressive behaviour	−0.8	0.319	−1.0	0.167	−0.1	0.867
Vocalisation (loud and soft)	24	0.299	30	0.192	16	0.524
Vocalisation loud	3	0.499	6	0.211	1	0.883
Vocalisation soft	19	0.092	10	0.374	10	0.397
Lip curl	0.2	0.549	1.0	0.020*	0.0	0.947
Rumination quality	−23	< 0.001***	−4	0.155	−11	< 0.001***
Eye white	−0.8	0.232	−0.2	0.760	−0.4	0.570
Activity budget (in s)						
Eating	−1304	< 0.001***	204	0.520	520	0.133
Rumination	−1351	0.002**	500	0.244	−709	0.127
Lying (left or right)	2460	< 0.001***	523	0.396	338	0.613
Lying left	144	0.756	−130	0.779	−826	0.103
Lying right	2317	< 0.001***	653	0.266	1152	0.071
Standing	−2283	< 0.001***	−341	0.585	151	0.822

*p < 0.05, **p < 0.01, **p < 0.001.

eating time on D1 (Table 6). Animals that calved via CS lay down more and spent less time standing on D1 in contrast with observations on D-30 ($p < 0.001$). When lying down, the right side was used more ($p < 0.001$). Time spent ruminating was less on D1 in comparison with that on D-30 ($p < 0.01$; Table 6).

Discussion

Comparison between the delivery *per vaginam* and CS group

The main differences in overall activity and activity budget between the two groups of cows were observed primarily on D1 (see Table 3 vs Table 4 and 5). Cows that underwent CS spent less time eating and ruminating, had more transitions in posture (from lying to standing or vice versa) and a longer resting time in comparison with naturally calving cows. Caesarean Section animals also showed less limb movements (and an associated tendency for lower overall activity) on D1, but this is presumably because of the highly significant difference in resting time.

The differences in eating and rumination time can be explained by the farm management as the farmer did not feed the CS animals during the first day after surgery to prevent adhesions between rumen and peritoneum. On D3, when food was available, eating time was higher in CS cows than in naturally calving cows, perhaps to compensate for the period of food deprivation. A higher frequency of transitions on D3 from standing to lying and vice versa could indicate an attempt to alleviate discomfort caused by pain. Alternatively, this behaviour could have increased because of a higher drive to forage. The higher resting time and decreased standing time in CS cows on the first day *post partum* can be interpreted as a probable pain indicator. Food deprivation cannot be invoked as an alternative explanation in this case, as food deprived cattle is motivated to spend more energy in their search for food (Schütz et al. 2006), which hence should decrease their time spent lying (Metz 1985).

On D1, the CS group laid down more on their right side ($p < 0.001$), but in contrast, this was not observed during the subsequent days and they did not lay down less on their left side (even at D1). This relative shift to the right side seems to indicate that the wound side is more painful.

Cows of the CS group reacted significantly more when pressure was put on the left flank on D1, D3 and D14, whereas naturally calving animals only showed more reaction on D1 when the area around the vulva was pressed. These results suggest that both parturition types provoke some pain and discomfort i.e. when the wound side for the CS group and the vulva area for the naturally calving group was squeezed. Pain after pressure apparently subsides faster in animals of the naturally calving group. Watts (2001) also used force applied onto the area around the surgical side to assess the severity of pain after CS and other surgery. Contrary to the results of the present study, Watts (2001) found no response to palpation after 60–72 h of surgery.

Comparison of the behaviour before vs after calving in both groups

There was a significant increase in the overall loud and soft vocalization on D14 in comparison to D-30 in the naturally calving group. This increase was not noticed in CS cattle. Previous research has indicated that vocalizations are an indicator of stress (Dunn 1990; Warris et al. 1994; White et al. 1995; Grandin 1998). In this study, each vocal sound was recorded including the sounds made towards the calf, loud and soft. Instead of vocalization by stress, the increase observed can also be because of a better dam-calf bonding in naturally calving cows. This hypothesis may also explain the decrease in aggressive behaviour towards the neighbour cow and her calf, seen in the naturally calving group.

Both groups showed less reaction to a loud noise on D1-14 compared with that on D-30. Although this could indicate depression in these animals, it can be suggested that it is more likely that animals got accustomed to the noise used in the experiment.

In the CS group, there are more highly significant ($p < 0.001$) differences in the time budget of D1 compared to that of D-30. Less time was spent eating and ruminating which was probably related to feed withdrawal. Reduced standing and increased lying time (principally on the right side may indicate reduced welfare (pain). The fast recovery to a normal pattern on D3 yet indicates that this effect was of a short duration. Compared to the period before parturition rumination quality in CS cows was decreased on D1, which is not surprising given the food deprivation. Yet, the CS cows also show a lower rumination quality on D14 in comparison with that on D-30, which is difficult to explain by the food deprivation alone. The reduced feed intake on D1 may also partially explain why CS animals have a lower (overall and specific) activity on D1 while this is not so for naturally calving animals. This can only be a partial explanation as overall activity (i.e. frequency of limb movements, ear flicking, etc.) on the third day *post partum* (D3) in CS cows is still significantly lower than basal levels (D-30), although the activity budgets i.e. time spent in different activities, had resumed basal levels (D-30) by then.

General discussion

The goal of this study was to examine objectively whether delivery per CS causes more pain and discomfort than delivery *per vaginam* in double muscled BB cows. The observations were restricted to only one species and furthermore, to one specific phenotype within one breed on one farm. It can therefore be assumed that observed behavioural differences are mainly because of individual variation and the experimental treatments. The choice of farm and animals was limited as the observations had to include naturally calving S-carcass BB cows. The authors have only been able to locate a single farm with a sufficient percentage of BB cattle that calves by vaginal delivery (30–50% annually vs the BB breed average of 0.15–0.5%). These limitations (small amount of animals and only one herd) may have caused some bias, but on the other hand, they minimize variation caused by genetic and management differences. Therefore, we assume that differences found in this study can be attributed to the method of parturition.

Conclusion

Our results indicate some statistically significant short-term behavioural differences between naturally calving and CS cows. Rumination quality, flank and vulva pressure and activity budget are the most likely parameters, indicating the discomfort in BB cows following naturally calving or CS. The practical circumstances under which this study was conducted (especially the management on D1) may partially explain the observed behavioural differences. Nevertheless, the results indicate an increased discomfort in cows that underwent CS.

It remains to be clarified whether the statistical differences indeed indicate biologically relevant differences in pain experiences and – thus – reduced welfare. In any case, this study shows that the behavioural differences observed in cows that underwent a correctly performed CS under local anaesthesia are predominantly subtle and of short duration.

Author contributions

Iris Kolkman, Hilde Vervaecke, Jo Vicca, Stefan Aerts and Jeroen VandeLook were the five observers. Dirk Lips, Geert Opsomer and Aart de Kruif helped with the discussion and made corrections of the article.

References

Bourne D, Cusdin P, Boardman SI, 2005: Pain management in Ruminants. CD-rom. Wildlife Information Network, East Midland Zoological Society, United Kingdom.

Cook CJ, 1997: Sex related differences in analgesia in sheep (Ovis ovis). N Z Vet J **45,** 169–170.

Cook CJ, 1998: Steroidal hormones determine sex-related differences in opioid-induced elevation of nociceptive threshold in sheep (Ovis aries). N Z Vet J **46,** 68–71.

Dunn CS, 1990: Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. Vet Rec **126,** 522–525.

European Community. 2003: Resolution of the Flemish Government about the organization of slaughtered adult cattle. 2003-10-03/37. Belgian Bulletin of Acts, Orders and Decrees.

Fitzpatrick JL, Nolan AM, Scott EM, Harkins LS, Barrett DC, 2002: Observer's perceptions of pain in cattle. Cattle Pract **10,** 209–212.

Flecknell PA, Waterman-Pearson A, 2000: Pain Management in Animals. W.B. aunders, London.

Grandin T, 1998: The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. Appl Anim Beh Sci **56,** 121–128.

Grandin T, 2001: Cattle vocalizations are associated with handling and equipment problems at beef slaughter plants. Appl Anim Beh Sci **71,** 191–201.

Hanset R, 2002: The Belgian White and Blue and the Caesarean Section: Can One Really Decrease the Frequency of the Caesarean Section in the Belgian White and Blue Without Losing its Typical Features. Publication Herd-Book du B.B.B. No 2002 03-27, Ciney, Belgium, 1–24.

Jourdan D, Ardid D, Eschalier A, 2001: Automated behavioural analysis in animal pain studies. Pharm Res **43,** 103–110.

Kolkman I, De Vliegher S, Hoflack G, Van Aert M, Laureyns J, Lips D, de Kruif A, Opsomer G, 2007: Caesarean section. Protocol of the caesarean section as performed in daily bovine practice in Belgium. Reprod Domest Anim **42,** 583–589.

Lay DC, Friend TH, Grissom KK, Bowers CL, Mal ME, 1992: Effects of freeze or hot-iron branding of Angus calves on some physiological and behavioural indicators of stress. Appl Anim Beh Sci **33,** 137–147.

Lips D, De Tavernier J, Decuypere E, Van Outryve J 2001: Ethical objections to caesareans: implications on the future of the Belgian White Blue. In: Proceedings of Eursafe 2001 "Food Savety, Food Quality and Food Ethics". The Third Congress of the European Society for Agricultural and Food Ethics, Florence, Italy, 3–5 October 2001.

Mellor DJ, Stafford K, 1999: Assessing and minimising the distress caused by painful husbandry procedures in ruminants. In Pract **9,** 436–446.

Mellor DJ, Molony V, Robertson IS, 1991: Effects of castration on behaviour and plasma cortisol concentrations in young lambs, kids and calves. Res Vet Sci **51,** 149–154.

Metz J, 1985: The reaction of cows to a short-term deprivation of lying. Appl Anim Beh Sci **4,** 301–307.

Mijten P, 1994: Complications of the Caesarean Section in Bovine. PhD Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 5–220.

Molony V, Kent JE, 1997: Assessment of acute pain in farm animals using behavioural and physiological measurements. J Anim Sci **75,** 266–272.

Molony V, Kent JE, Robertson IS, 1995: Assessment of acute and chronic pain after different methods of castration of calves. Appl Anim Beh Sci **46,** 33–48.

Morton DB, Griffiths PHM, 1985: Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. Vet Rec **20,** 431–436.

Phillips C, 2002: Cattle Behaviour and Welfare. Blackwell Science Ltd, Oxford.

Queyras A, Carosi M, 2004: Non-invasive techniques for analysing hormonal indicators of stress. Ann Ist Super Sanita **40,** 211–221.

Sandem AI, Braastad BO, Bøe KE, 2002: Eye White may indicate emotional state on a frustration-contentedness axis in diary cows. Appl Anim Beh Sci **79,** 1–10.

Sandem AI, Braastad BO, Bakken M, 2005: Behaviour and percentage eye-white in cows waiting to be fed concentrate – a brief report. Appl Anim Beh Sci **95,** 233–239.

Sanford J, Ewbank R, Molony V, Tavernor WD, Uvarov O, 1986: Guidelines for the recognition and assessment of pain in animals. Vet Rec **118,** 334–338.

Schütz K, Davison D, Matthews L, 2006: Do different levels of moderate feed deprivation in dairy cows affect feeding motivation? Appl Anim Beh Sci **101,** 253–263.

Warris PD, Brown SN, Adams SJM, 1994: Relationship between subjective and objective assessments of stress at slaughter and meat quality in pigs. Meat Sci **38,** 329–340.

Watts S, 2001: Aspects of Analgesia in Cattle. Phd. University of London, London, 59.

Webster AJF, 2002: Rendering unto Caesar: welfare problems in Belgian Blue Cattle. Vet J **163,** 228–229.

White RG, DeShazer JA, Tressler CJ, Borcher GM, Davy S, Waninge A, Parkhurst AM, Milanuk MJ, Clemmens ET, 1995: Vocalizations and physiological response of pigs during castration with and without local anesthetic. J Anim Sci **73,** 381–386.

Submitted: 20 Sep 2008

Author's address (for correspondence): Iris Kolkman, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium. E-mail: iris.kolkman@ugent.be