



Quantifying subclinical trauma associated with calving difficulty, vigour, and passive immunity in newborn beef calves

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To cite: Pearson JM, Homerosky ER, Caulkett NA, *et al.* Quantifying subclinical trauma associated with calving difficulty, vigour, and passive immunity in newborn beef calves. *Veterinary Record Open* 2019;6:e000325. doi:10.1136/vetreco-2018-000325

Preliminary results were presented at: the Canadian Association of Veterinary Epidemiology and Preventive Medicine Conference, Calgary, Alberta, June 2017; the Canadian Beef Industry Conference, Calgary, Alberta, August 2017; and the American Association of Bovine Practitioners Annual Conference in Omaha, Nebraska, September 2017.

Received 23 October 2018
Revised 7 December 2018
Accepted 14 December 2018

ABSTRACT

This cross-sectional study quantifies subclinical trauma associated with calving difficulty, calf vigour, and passive immunity (PI) in newborn beef calves. The degree of calving difficulty was categorised as: unassisted, easy assist (one or two people manually pulling to deliver the calf) and difficult assist (more than two people pulling, a fetal extractor (ie, calf jack), or caesarean section). Vigour assessment occurred at 10 minutes and blood sampling at 24 hours after birth in 77 beef calves. The measured blood parameters associated with trauma were creatine kinase (CK), aspartate aminotransferase (AST), and haptoglobin. Serum IgG concentration was measured, and an IgG concentration at least 24 g/l was considered as adequate PI. Calving difficulty was associated with elevated levels of CK ($P=0.002$) and AST ($P=0.01$), weak suckle reflex ($P=0.001$), abnormal mucous membrane colour ($P<0.0001$), and decreased odds of adequate PI ($P=0.004$). Elevated levels of CK and AST were associated with abnormal mucous membrane colour, incomplete tongue withdrawal and weak suckle reflex at birth ($P<0.001$). An incomplete tongue withdrawal ($P=0.005$) and weak suckle reflex ($P=0.02$) were associated with decreased IgG concentrations. Abnormal mucous membrane colour, incomplete tongue withdrawal, and a weak suckle reflex were associated with decreased odds of having adequate PI ($P<0.05$). Haptoglobin was not associated with any of the parameters measured. Subclinical trauma was associated with calving difficulty, decreased vigour and decreased odds of having adequate PI. Understanding the impacts of a traumatic birth may aid the development of management strategies for compromised newborn beef calves.

INTRODUCTION

Calf health and survival is crucial to successful cow-calf operations. Although the majority of producers rank calving ease and birth weight as their most important selection criteria when purchasing replacement bulls,¹ approximately 9 per cent of calvings are assisted in western Canada.² This proportion fluctuates between herds and between different years, but almost all cow-calf operations have some calvings that require assistance each season.

Calves that are assisted at birth are more likely to experience trauma or oxygen deprivation during the birthing process, which may negatively influence their vigour.³⁻⁵ Trauma caused by an assisted calving includes: fractured or luxated vertebrae, ribs and legs; crushing injuries; vascular compromise to soft tissues; and oedema of the tongue or head due to prolonged periods in the dam's pelvic canal.^{6,7} Assisted calves also have a higher risk of acidaemia and hypoxia, which can lead to decreased vigour and decreased transfer of colostrum.⁸⁻¹⁰ Failed transfer of passive immunity (PI) is caused by the inadequate ingestion or absorption of enough good-quality colostrum¹¹ and is associated with increased preweaning morbidity and mortality.^{12,13}

Higher risk of stillbirth and preweaning morbidity and mortality are consequences of difficult calvings.^{2,14,15} However, the extent or severity of trauma, and the impacts of that trauma on calf wellbeing have not been well described. Unless the trauma is obvious (eg, fractures), on farm this trauma often goes undiagnosed and therefore, untreated.¹⁶ The degree of tissue damage, disease, or organ malfunction is commonly assessed in veterinary practice based on certain biochemical parameters measured in blood or other tissues. Muscle trauma, inflammation, or other muscular pathological processes can be measured by serum creatine kinase (CK) and aspartate aminotransferase (AST). These enzymes are released into the plasma after muscle or other organ damage has occurred.¹⁷ Currently, there is no published research quantifying the relationship of these blood parameters with the trauma of calving difficulty.

Therefore, the objective of this study was to quantify subclinical trauma, as measured by



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elevated muscle enzymes and haptoglobin, a marker for inflammation, and its association with calving difficulty, vigour assessment parameters, and serum IgG concentration. It was hypothesised that elevated levels of CK, AST, and haptoglobin would be associated with greater calving difficulty, reduced vigour, and failed transfer of PI, and calves with reduced vigour were suspected to have increased odds of failed transfer of PI.

MATERIALS AND METHODS

The data were collected in March 2014 in Alberta, Canada from 77 calves that were enrolled as described previously.^{18 19} A privately-owned cow-calf operation located in southern Alberta was enrolled as the study herd. It consisted of approximately 800 Hereford and Hereford x Red Angus cows and heifers. Animals were enrolled using a purposive sampling regime to represent both mature cows and heifers and include unassisted (UA) and assisted calvings. Pregnant dams were monitored in small pastures close to the calving barn and checked hourly for signs of calving. Unassisted (UA) dams were allowed to calve without assistance in the pasture, and the calves were brought into the barn within 10 minutes of birth. Failure to calve or make progression within one hour of estimated onset of stage 2 labour (eg, amniotic sac visible, feet present, strong abdominal contractions, etc) resulted in the dam being walked to the nearby calving barn for vaginal examination and assisted delivery of the calf. If the calf was unable to be delivered vaginally, a veterinarian examined the dam, and if necessary, a caesarean section (C-section) was performed.

At birth, data that were collected included: date and time of calving, calving ease score, presentation and posture of the calf, dam parity, calf sex, and calf birth weight. The subjective measure of calving ease score was categorised as UA, easy assist (EA), or difficult assist (DA). Easy assists (EA) were defined as one or two people manually pulling to extract a calf. Difficult assists (DA) were those that required more than two people, a fetal extractor (ie, calving jack; Dr Franks Calf Puller, Neogen), or C-section. The presentation of the calf was defined as anterior versus posterior, and any abnormal posture (eg, head ventroflexed, leg malpositioned, etc) was also recorded for assisted calvings.

Within 10 minutes of birth, all calves were placed in sternal recumbency and evaluated for vigour parameters. The vigour parameters used in this study were examined by Homerosky and colleagues^{18 19} and were associated with acidaemia (mucous membrane colour and tongue withdrawal) and likelihood of standing to nurse without human assistance within four hours of birth (suckle reflex). Mucous membrane colour was categorised into pink (light pink or dark pink) or abnormal (blue-purple, white, dark red) by visual examination of the oral mucous membranes. Tongue withdrawal was categorised as complete or incomplete depending on whether the calf withdrew the tongue back into its mouth fully or not

when the tongue was pulled from its mouth. Suckle reflex was categorised as strong or weak by placing a finger in the calf's mouth and feeling if it suckled the finger. Calves born dead or that did not survive to 10 minutes were not enrolled.

After vigour assessments were performed, cow-calf pairs were placed in individual box stalls and monitored for colostrum consumption. If calves were not observed nursing from the dam by four hours after birth, research personnel intervened with colostrum consumption first by assisting the calf to nurse from the cow in a chute. If the calf did not nurse from the dam, the dam was hand-milked and the calf bottle or tube fed 0.5–1.25 litres of colostrum by six hours, as per on-farm protocols.

A blood sample was collected from each calf by jugular venipuncture at approximately 24 hours after birth¹⁸ using a 10 ml silicon-coated serum separator vacutainer tube and 20 G x 2.54 cm needle (BD Vacutainer). Whole blood samples were stored at 4°C until processed. Within 12 hours of collection, samples were centrifuged at 1400 G for 20 minutes. Serum was extracted and frozen at –80°C until further analysis.

Biochemistry profile analysis was performed on 24-hour post-birth serum samples using a Beckman AU680 chemistry panel machine (Beckman/Coulter) at the IDEXX Reference Laboratories (Calgary, AB). Haptoglobin analysis was performed by photometric analysis using the 6000c501 biochemistry analyser (Roche Cobas) at the Animal Health Laboratory, University of Guelph (Guelph, ON). Serum IgG concentrations were also measured in the 24-hour serum samples using an in-house radial immunodiffusion assay at the Saskatoon Colostrum Quality Assurance Laboratory (Saskatoon, SK), as described by Chelack *et al.*²⁰

Data were analysed using STATA V.14.1 software (StataCorp). Descriptive statistics and tests for normality were performed on all continuous variables. Adequate PI was categorised as serum IgG concentration above a cut-point of 24 g/l.²¹ To assess differences between proportions of assisted heifers and cows, a Fisher's exact test and pairwise comparisons were performed. To evaluate the association of calving ease score with the continuous blood parameters indicative of trauma and serum IgG concentration, Kruskal-Wallis tests were performed on the non-parametric outcomes (CK, AST, and haptoglobin) and one-way analysis of variance was performed on the parametric outcome (serum IgG concentration). To determine the association of calving ease score on the categorical outcomes of vigour and adequate PI, a Fisher's exact test was used. To assess the association of blood parameters indicative of trauma on the categorical outcomes of vigour, Wilcoxon rank-sum tests were performed. To evaluate the association of vigour on serum IgG concentration, a Student's *t* test was performed, and a Fisher's exact test was performed to evaluate the association of vigour with adequate PI. Pairwise comparisons between calving ease score, vigour parameters, and adequate PI were done using a Bonferroni correction

**Table 1** Descriptive statistics of 77 cow-calf pairs by calving ease score

Variable	Unassisted*	Easy assist†	Difficult assist‡
Dam			
Heifers (%)	8 (29.6)	9 (33.3)	10 (37.0)
Cows (%)	14 (28.0)	32 (64.0)	4 (8.0)
Calf sex			
Bull (%)	5 (13.9)	24 (66.7)	7 (19.4)
Heifer (%)	17 (41.5)	17 (41.5)	7 (17.0)

*Calf delivered without assistance at birth.

†One or two people pulling to extract a calf.

‡More than two people pulling, a fetal extractor or caesarean section used to extract a calf.

test. Odds ratios (OR) were calculated; however, when a cell had a zero for its count, 0.5 was added to all cells for the calculation.²²

RESULTS

Data were collected from calves born to 50 mature cows (65 per cent) and 27 heifers (35 per cent). Forty-one heifer calves (53 per cent) and 36 bull calves (47 per cent) were enrolled. As reported previously, there was no difference between the proportion of total assisted births between heifers and cows, nor in the average birth weight between bull and heifer calves.¹⁸ A heifer was more likely to be a DA than an EA compared with cows ($P=0.0008$), but the proportions did not differ between the other groups ($P>0.06$). The majority of calves were born in anterior presentation (91 per cent) and normal posture (84 per cent). The sampled population categorised by calving ease score is reported (table 1). Median birth weight and IQR by calving ease score was 38.4 kg (37.5–41.0) for UA, 40.7 kg (36.4–45.7) for EA, and 39.7 kg (36.1–43.3) for DA.

Twenty-two calves were categorised as UA (28.6 per cent), 41 as EA (53.2 per cent), and 14 as DA (18.2 per cent), as previously reported.¹⁸ Two of the 14 DA calves categorised as DA were born via C-section. They

were categorised as a DA because there were extensive efforts made by the farm personnel to deliver the calves vaginally prior to surgical intervention by a veterinarian. A DA was associated with elevated CK and AST levels as compared with EA and UA (table 2). There was no association between calving ease score and haptoglobin levels or serum IgG concentrations (table 2). Calving ease score had an effect on suckle reflex and mucous membrane colour. A DA was associated with a higher proportion of calves with weak suckle reflexes when compared with UA (OR 45.0, $P=0.0004$) and EA (OR 4.9, $P=0.02$). A DA was associated with a higher proportion of calves with abnormal mucous membrane colour when compared with UA (OR 45.0, $P=0.0004$) and EA (OR 9.3, $P=0.02$). Twenty-two (100 per cent) UA calves, 38 (92.7 per cent) EA calves and nine (62.3 per cent) DA calves had adequate PI. Difficult assists were associated with a higher proportion of calves with inadequate PI when compared with UA (OR 26.1, $P=0.005$) and EA (OR 7.04, $P=0.019$).

An abnormal mucous membrane colour at birth was associated with significantly elevated CK and AST levels (table 3). An incomplete tongue withdrawal and weak suckle reflex were associated with elevated CK, AST, and decreased serum IgG concentrations (table 3). Haptoglobin was not associated with any vigour parameters (table 3). Abnormal vigour parameters (abnormal mucous membrane colour, incomplete tongue withdrawal, and weak suckle reflex) were associated with higher odds of inadequate PI ($P<0.05$) (table 4).

DISCUSSION

In the present study, DA calvings were associated with increased tissue trauma, as evidenced by elevated CK and AST levels, higher odds of reduced vigour, and higher odds of inadequate PI, indicating that a more difficult birth causes more tissue trauma and impacts calf vigour and PI. Although many textbooks may report that difficult calvings cause trauma, it has not been fully investigated nor quantified. Undiagnosed trauma is often observed at necropsy in the form of subcutaneous bruising.^{3,23} In the present study, no fractures or obvious

Table 2 Descriptive statistics for blood parameters indicative of trauma and serum IgG concentrations measured at 24 hours by calving difficulty in 77 cow-calf pairs*

Blood parameter	Unassisted†	Easy assist‡	Difficult assist§	P value
CK (iu/l)¶	233.5 (192–343)	310 (228–458)	696 (268–1441)	0.002
AST (iu/l)¶	61.5 (55–73)	71 (59–82)	78 (63–119)	0.01
Haptoglobin (g/l)¶	0.15 (0.13–0.16)	0.14 (0.13–0.16)	0.14 (0.13–0.15)	0.9
Serum IgG (g/l)**	47.4 (42.2–52.6)	43.3 (39.3–47.4)	36.6 (24.6–48.7)	0.1

*Pairwise comparisons reported in text.

†Calf delivered without assistance at birth.

‡One or two people pulling to extract a calf.

§More than two people pulling, a fetal extractor or caesarean section used to extract a calf.

¶Median (first interquartile to third interquartile) reported for non-normally distributed variables.

**Mean (95% CI) reported for normally distributed variables.

AST, aspartate aminotransferase; CK, creatine kinase.

Table 3 Descriptive results for blood parameters indicative of trauma and serum IgG concentration by vigour parameters in 77 cow-calf pairs

Variable	Categories		P value
Mucous membrane colour	Pink (n=66)	Abnormal (n=11)	
CK (iu/l)*	270 (218–401)	878 (514–4178)	<0.0001
AST (iu/l)*	67 (57–78)	94 (71–130)	0.002
Haptoglobin (g/l)*	0.14 (0.13–0.15)	0.14 (0.13–0.19)	0.3
Serum IgG (g/l)†	44.4 (41.5–47.5)	36.0 (20.0–51.9)	0.07
Tongue withdrawal	Complete (n=60)	Incomplete (n=15)	
CK (iu/l)*	270 (212.5–411.5)	584 (309–1441)	0.0004
AST (iu/l)*	67 (55.5–77.5)	82 (76–119)	0.0009
Haptoglobin (g/l)*	0.14 (0.13–0.16)	0.15 (0.13–0.16)	0.4
Serum IgG (g/l)†	45.5 (42.0–49.0)	33.8 (25.2–42.4)	0.005
Suckle reflex	Strong (n=63)	Weak (n=14)	
CK (iu/l)*	269 (206–420)	544 (309–1441)	0.0005
AST (iu/l)*	67 (55–77)	88.5 (76–130)	0.0002
Haptoglobin (g/l)*	0.14 (0.13–0.16)	0.135 (0.13–0.16)	0.9
Serum IgG (g/l)†	45.0 (41.7–48.3)	35.3 (24.7–45.9)	0.02

*Median (first interquartile to third interquartile) reported for non-normally distributed variables.

†Mean (95% CI) reported for normally distributed variables.

AST, aspartate aminotransferase; CK, creatine kinase.

signs of trauma were diagnosed at birth, but the calves experiencing a DA had elevated levels of blood parameters associated with subclinical trauma. Subclinical trauma caused by a difficult birth may be more prevalent than previously recognised. A randomised clinical trial for a viral respiratory vaccine unexpectedly found a prevalence of 6 per cent rib fractures in dairy calves during thoracic ultrasonography.¹⁶ Those authors found that these undiagnosed rib fractures were associated with assisted calving and with decreased average daily gain.¹⁶

Table 4 Descriptive results for vigour parameters by passive immunity in 77 cow-calf pairs

Variable	Passive immunity		OR	P value
Mucous membrane colour	Inadequate* (n=8)	Adequate† (n=69)		
Abnormal (n=11)	5	6	17.5	0.001
Pink (n=66)	3	63		
Tongue withdrawal	Inadequate* (n=8)	Adequate† (n=67)		
Incomplete (n=15)	4	11	5.1	0.05
Complete (n=60)	4	56		
Suckle reflex	Inadequate* (n=8)	Adequate† (n=69)		
Weak (n=14)	4	10	5.9	0.03
Strong (n=63)	4	59		

*Inadequate passive immunity (serum Ig concentration <24 g/l).

†Adequate passive immunity (serum Ig concentration ≥24 g/l).

Therefore, subclinical trauma may have long-term effects on calf health and production.

Calves that experienced a DA in this study had significantly elevated CK and AST values compared with UA and EA calves. Serum CK is a sensitive and specific biochemical indicator that can detect subclinical muscle injury and trauma.²⁴ The half-life of serum CK is approximately four hours and values decrease rapidly if the cause of muscle damage ceases.^{25 26} In contrast, AST has a half-life of about 20 hours and changes more slowly than CK.²⁷ Therefore, subclinical trauma experienced at birth can be detected at 24 hours of age using a combined assessment of CK and AST.¹⁷ Few studies have quantified serum biochemical results in newborn calves and those authors found elevated CK levels at birth that declined over time.^{28 29} They hypothesised these findings were due to trauma associated with birth, but they did not specifically investigate the association with varying degrees of calving difficulty. The present study demonstrated subclinical trauma was associated with difficult births as measured by elevated levels of CK and AST, and this differed from EA and UA births.

Subclinical trauma was associated with reduced vigour, and serum IgG concentrations were significantly lower in calves with weak suckle reflexes and an incomplete tongue withdrawal. Various assessment of newborns, such as the Apgar score, has been used in various neonatal species to classify their vigour at birth.^{30–32} A difficult birth can result in a less vigorous neonate, which subsequently leads to a prolonged time to stand and time to suckle from the dam.^{33–35} The present study is consistent with

other studies demonstrating that calves with prolonged or difficult calvings had reduced vigour compared with calves assisted earlier during the parturition process³⁶ or UA calvings.³⁷ Reduced vigour and vitality have been shown to be associated with acidaemia, hypoxia, and elevated L-lactate.^{5 18} Acidaemia or hypoxia could be another physiological explanation for reduced vigour in assisted calves. The relationship between subclinical trauma and hypoxia as a result of a difficult calving was not investigated but should be investigated in future studies.

Reduced vigour has also been associated with inadequate PI in beef and dairy calves.^{19 38} A previous analysis performed on this sampled population showed that calves with a weak suckle reflex had higher odds of failing to consume colostrum on their own by four hours, and that there was an association between those that failed to consume colostrum by four hours and inadequate transfer of PI.¹⁹ Vasseur *et al*³⁹ found that a dairy calf's overall vigour (defined as attempting to stand within one hour of birth) was associated with colostrum intake. They also found birth weight, vigour during feeding, and vigour during the first hour of life were associated with the quantity of colostrum ingested. Although the outdoor birthing environment for UA calves was different from the indoor birthing environment of EA and DA calves, there was no difference in vigour parameters between UA and EA calves. This suggests environmental factors had a negligible effect on calf vigour in this study.

In this study, calving ease score was not associated with the continuous outcome of serum IgG concentration, but it was significantly associated with the dichotomised outcome of adequate PI. This may indicate that even if the mean concentration of Ig is not significantly different by calving ease score, a difficult calving still influences the likelihood of a calf reaching a threshold of adequate PI. The purpose of using a standard cut-off for serum IgG concentration is to determine which calves are at an increased risk for morbidity and mortality.²¹ Although no association between calving ease score and serum IgG concentration was reported in this study, others have reported a decrease in serum IgG concentration with increasing calving difficulty.^{38 40 41} Our lack of significant differences may be due to a small proportion of calves having low concentrations of IgG, as all calves received colostrum by six hours after birth. One similar study found no association between calving difficulty and serum IgG concentrations when those calves were all fed one litre of colostrum shortly after birth.⁴² A limitation to this study was the variation in the quality, volume, and route of colostrum consumption. It is well documented that high-quality colostrum and larger concentrations of colostrum IgG fed to dairy calves improve PI,⁴³ but that was not standardised in this study due to on-farm protocols.

Haptoglobin did not differ significantly among calving ease scores, nor was it associated with reduced vigour. Haptoglobin is one of the major acute-phase proteins in cattle⁴⁴ and is released in the early stages of inflammation

and infection or after tissue damage.^{45 46} Although acute-phase proteins are not specific to a certain disease, they are quite sensitive to inflammation and can detect subclinical disease in some species.⁴⁷ Similar to the results of the present study, Asemgeest *et al*⁴⁸ reported that haptoglobin was undetectable or in low concentrations in newborn calves after different types of obstetrical help. They suggested that the trauma of parturition did not increase acute-phase proteins in neonates due to the inability of the immature liver to produce the protein.⁴⁸

Although the primary objective of this study was achieved, there were limitations. One is the lack of control over variables in the environment and potential confounders that exist on a commercial ranch. However, this is balanced with the increased external validity of using such a ranch compared with a research facility. It was not feasible to select at random the animals in this study. They were enrolled based on a purposive sampling of cattle calving within a 24-day period and that could be handled without injury to the animal handlers or excessive stress to the periparturient dam. On this ranch, the animals were intensely managed. Early intervention to increase the proportion of live-born calves may have inflated the number of calves in the easy assisted group and underestimated the UA group, if those animals had been given more time to calve. Interestingly, only DAs, which likely represent 'true dystocias', had significantly elevated blood parameters associated with trauma, reduced vigour, and increased odds of inadequate PI. In situations where the dam's birth canal is fully dilated, early intervention may indicate a positive management decision to aid in the prevention of calves becoming compromised due to a prolonged calving.³⁶ Although not assessed in this study, it is important to note that the degree of interaction between the dam and the calf may influence the vigour, time to stand, and time to nurse in neonatal calves.^{49 50} Also, further studies to investigate pain and inflammation associated with difficult calvings are warranted as this may impact calf vigour and health.

CONCLUSIONS

Difficult births lead to elevated indicators of subclinical trauma and decreased vigour in the neonate, which can be quantified by measuring serum CK, AST, and vigour parameters, respectively. Trauma and reduced vigour can lead to inadequate transfer of PI. These findings suggest further studies are needed to investigate appropriate management practices to decrease the impacts of a difficult calving on calf health.

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Acknowledgements The authors sincerely thank Drs Grace Kwong and Soren Boysen for their time, guidance and/or laboratory equipment, Dr Christine Murray, Maggie Grover, Danielle Cyr and Sarah van Schothorst for their assistance in data collection, and the participating ranch for making this study possible. The authors also thank the Saskatoon Colostrum Company Ltd. for their in-kind contribution to analysing serum immunoglobulins.

Funding This study was supported by the Simpson Ranch Beef Cattle Health Endowment and the University of Calgary Faculty of Veterinary Medicine Eyes High Scholarship programme.

Competing interests None declared.

Ethics approval The study was approved on February 3, 2014 by the University of Calgary Veterinary Sciences Animal Care Committee (AC13-0324) and was conducted in accordance with the guidelines established by the Canadian Council on Animal Care.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The confidentiality agreement with the participating beef producer precludes sharing this data.

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