Associations of serum haptoglobin in newborn dairy calves with health, growth, and mortality up to 4 months of age

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ABSTRACT

The objective of this research was to investigate factors associated with serum haptoglobin (Hp) levels in newborn calves. In addition, the associations between serum Hp levels in newborn calves with growth, morbidity, and mortality in calves <4 mo of age were investigated. A total of 1,365 Holstein heifer calves from 15 dairy farms were enrolled in this study from January to December, 2008. Following calving, a birth record was completed, including information on the calving event, colostrum administration, and other details. During weekly farm visits, each calf was assessed at 1 to 8 d, 15 to 21 d, 36 to 42 d, and 90 to 120 d of age. At these sampling times, each calf was assessed using a standardized clinical score for general health, and height and weight were measured. At 1 to 8 d of age, a blood sample was collected to measure serum total protein and Hp concentrations. Treatment events and death loss were recorded throughout the study by the farm staff. Serum Hp concentration in the first week of life was not significantly associated with the degree of calving difficulty. However, serum Hp was higher in calves with a higher rectal temperature and depressed attitude at the first sampling time. Furthermore, the association between serum Hp and the severity of nasal discharge varied by age at first sampling time. Calves with higher Hp in their first week of life had significantly higher total health scores throughout the entire sampling period. Haptoglobin was not significantly associated with average daily gain or treatment for bovine respiratory disease. Yet, for every 1 g/L increase in serum Hp in the first week of life, the odds of being treated for any other disease during the study period increased by 7.6 times. Treatment for bovine respiratory disease, diarrhea, or any other disease resulted in increased odds of calf mortality. In addition, Hp concentration in the first week of life was associated with mortality in calves <4 mo of age. The optimal cut point for Hp was de-

termined to be 0.13 g/L for the prediction of disease and death, although the sensitivity of Hp concentration alone as a diagnostic test for individual calves was low. **Key words:** haptoglobin, calving difficulty, health, mortality

INTRODUCTION

Infectious diseases are a serious issue on commercial dairy farms, and can lead to substantial economic loss and animal welfare concerns. Tools for the diagnosis and surveillance of disease are needed to prevent these losses (LeBlanc et al., 2006).

Blood biochemical and hematological values are gaining broader attention for their potential role in disease diagnosis and as a measurement of general health. Specifically, acute-phase proteins produced in response to a bacterial or viral challenge can be used as quantitative indicators of infection, inflammation, tissue injury, or stress (Murata et al., 2004; Petersen et al., 2004). One of the major acute-phase proteins in the bovine is haptoglobin (Hp), which reduces pro-oxidative and proinflammatory stress through binding hemoglobin and removing it from circulation (Murata et al., 2004; Petersen et al., 2004). In addition, it has been shown that Hp has higher sensitivity in detecting disease compared with other acute-phase proteins, due to its more pronounced and prolonged response to infection (Angen et al., 2009).

In calves, disease has been associated with significant increases in serum Hp concentration (Heegaard et al., 2000; Gånheim et al. 2007; Angen et al., 2009), which can increase over 100 fold upon immune stimulation (Conner et al., 1988, 1989). Thus, researchers have suggested that serum Hp concentrations could be used to assess inflammation and disease status in calves (Carter et al., 2002; Angen et al., 2009). Combining serum Hp with rectal temperature may be a valuable parameter for herd-level diagnostics in heifer calves (Svensson et al., 2007).

Few research studies exist that have investigated the effects of difficulty at parturition on Hp levels in newborn calves. Yet, it is logical to assume that serum Hp levels would increase in newborn calves following

Received June 9, 2014.

Accepted August 14, 2014.

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a difficult calving when excessive force during extraction causes fetal stress and trauma (Haughey, 1975; Schuijt, 1990). Some reports have shown that neonatal Hp levels are high following birth (Knowles et al., 2000; Orro et al., 2008), but did not investigate the effect of calving difficulty on Hp concentrations. It has also been suggested that a difficult birth can increase the risk of morbidity and mortality later in life (Schuijt and Taverne, 1994; Lombard et al., 2007). Thus, if Hp concentration increases in association with calving difficulty, it is possible that it could be used as a biomarker for increased risk of morbidity and mortality.

The objective of this research was to investigate factors associated with serum Hp levels in newborn calves. In addition, the associations between newborn serum Hp levels with future growth, morbidity, and mortality in calves <4 mo of age were investigated.

MATERIALS AND METHODS

Study Farms and Animals

Newborn heifer calves from 15 commercial dairy farms in southwestern Ontario were enrolled in this study. Farms were selected with the assistance of local dairy practitioners and were included in the study based on criteria of farm accessibility (within 250 km of the University of Guelph in Guelph, ON, Canada) and reliable record keeping. Participating farms were not requested to change any aspects of their calf management for this study. All heifer calves born on the farms during the sampling period were included in the study. The Animal Care Committee at the University of Guelph approved the study.

Study Enrollment and Sampling

During the period from January to December 2008, heifer calves were enrolled between 1 and 8 d of age. Calves were uniquely identified with National Livestock Identification for Dairy tags (Brantford, ON, Canada) at each farm.

Producers were requested to complete a birth record for each heifer calf using forms that were provided. The birth record detailed information about the delivery circumstances, calving environment, colostrum practices, and perinatal treatments for each calf.

Farms were visited weekly by research technicians. At first sampling (1 to 8 d), a blood sample was collected by jugular venipuncture from each calf. Using a 20-gauge, 2.5-cm hypodermic needle, 10 mL of blood was drawn into a sterile glass serum collection tube without anticoagulant (BD Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). The blood samples

were stored in coolers with an ice pack and transported to the laboratory for processing.

Calves were examined at 4 sampling times: 1 to 8 d, 15 to 21 d, 35 to 42 d, and 90 to 120 d of age. Height, weight, and rectal temperature were measured and a health score was assigned. Height was measured at the withers with the head in a neutral position. Weight was measured using a heart-girth measuring tape pulled snugly around the thorax, just caudal to the forelimb (calves up to 5 wk: Dairy Calf Tape, and calves between 90 to 120 d: Weight-By-Breed Dairy Cow Tape; The Coburn Co. Inc., Whitewater, WI). Rectal temperature was determined using a digital thermometer (Omron Healthcare Inc., Burlington, ON, Canada). Health scores for attitude, ear position, nasal discharge, and fecal consistency using a scoring system adapted from the Calf Health Scoring Criteria previously published by the University of Wisconsin (Madison) were measured (McGuirk, 2008). Five-point scales were used for attitude and ear position, and 4-point scales were used for nasal discharge and fecal consistency. Descriptions and photographs for each score were provided to the research technicians and participating farms as a means of standardizing these assessments.

During the monitoring phase from enrollment to 90 to 120 d of age, farmers were requested to record all treatments and mortality in heifer calves using the forms supplied. For every treatment administered, the producer recorded the date, suspected disease using a standardized case definition (Windeyer et al., 2014), rectal temperature, and treatment given. To be included, the treatments must have been given because of suspected illness, not as a preventative measure. Treatments with a long-acting antibiotic had to be more than 7 d apart to be considered as new treatment events. For any other medications, treatments had to be more than 3 d apart to be considered as separate treatment events.

A farm-level questionnaire was administered to record general information about the farm operation and protocols, including details of the barn facilities and capacity, maternity areas, calf housing, calf feeding equipment, vaccination, deworming, dehorning, calving procedures, and weaning protocols.

Blood Analysis

Blood samples were centrifuged at $970 \times g$ for 10 min at approximately 20°C within 24 h of collection. Serum was separated and used to assess serum total protein (STP) concentration by digital refractometry (range: 1–12g/dL; accuracy: ± 0.1 ; model 300027; Sper Scientific Ltd., Scottsdale, AZ). Aliquots of serum were stored at -20°C and subsequently analyzed for

Hp using a photometric automated analyzer (range: 0–0.9 g/L; Hitachi 911 Analyzer; Roche Diagnostics, Laval, QC, Canada).

Statistical Analysis

All data were entered into Microsoft Access software (Microsoft Corp., Redmond, WA) and exported into Stata-IC 10.1 software (StataCorp, College Station, TX) for variable screening and statistical modeling. Calf-level explanatory variables that were offered to statistical modeling included calving factors (location, assistance, malpresentation, twins, time of day, month, and season), colostrum management (timing, source, type, and method of delivery), perinatal treatments (navel dip, vitamin or mineral injections, antibiotics, vaccination, or supplemental antibody products), age at enrollment, STP or passive transfer status (fail: <5.2 g/dL; intermediate: 5.2 to 5.4 g/dL; pass: >5.4 g/dL), rectal temperature, BW and calf health scores, treatment for disease [bovine respiratory disease (**BRD**), diarrhea, or other diseases such as omphalophlebitis or arthritis], and mortality. Seasons were defined as winter from December through February, spring from March through May, summer from June through August, and fall from September through November. Herd-level explanatory variables included herd characteristics (size, region, and barn design), cow and calf vaccination protocols, preweaning housing (type, location, ventilation, bedding, cleaning frequency, number of calves, and shared airspace), milk feeding (type, method of delivery, and volume), weaning (age when starter and hay offered, basis and age of weaning, and average weight) and postweaning housing (type, location, ventilation, bedding, cleaning frequency, number of calves, shared airspace, and age at dehorning).

A causal diagram was constructed to examine the possible relationships to be tested among variables. All variables were examined with descriptive statistics to determine the distribution of the data, and to look for missing values. If abnormal values were detected, data were checked against the original hard copy of data to ensure accuracy.

Univariable Regression Models. Univariable associations between serum Hp concentration and several predictor variables, including calving assistance, season of birth, days of age at the first sampling time (1 to 8 d), passive transfer, and health score at the time of first sampling, were assessed graphically and as mixed linear models with farm as the random intercept. In addition, univariable mixed logistic models were constructed between serum Hp concentration with the following outcome variables: treatment for BRD, treatment for any other disease including diarrhea, and mortality.

Mixed Multivariable Linear Regression Models. Three separate mixed multivariable linear regression models were constructed using the `xtmixed` command in Stata software, with farm as a random intercept. Models were built to assess whether statistically significant associations existed between previously listed predictor variables and the following outcomes: Hp concentration, total health score, and ADG.

All predictor variables hypothesized to be related to the outcome of interest were screened for unconditional associations in univariable analyses, which included farm as a random intercept. Any predictor variables that were liberally associated with the outcome ($\alpha = 0.2$) were considered for inclusion in the final model. Because Hp concentration was the variable of interest, it was forced into each model, even if it was not statistically significant. Continuous variables were assessed for linearity with the outcome variable using locally weighted regression (lowess smoothers). If a variable was nonlinear, a quadratic term was constructed and tested in the model. If the quadratic term was significant ($\alpha = 0.05$) in the model, and the relationship was adequately modeled using a curve, the quadratic term was retained in the model. If not, the quadratic term was removed and logarithmic and square root transformations were assessed for linearity and significance. If none of the presented transformations appeared to improve linearity, the variable was categorized.

Spearman correlation coefficients were calculated for all predictor variables that were considered for inclusion in the multivariable model to detect issues of collinearity. Consequently, if the correlation coefficient was greater than $|0.8|$, the variable that made the most biological sense or had the fewest missing observations was used. Confounding was assessed by backward elimination. A confounding variable was defined as a nonintervening variable whose removal resulted in a >25% change in the coefficients of significant variables. Regardless of the *P*-value, confounding variables were retained in the model. Predictor variables in each model were checked for interaction by including all biologically appropriate 2-way interaction terms identified in the causal diagram, one at a time, into a main effects model. Statistically significant interaction terms ($\alpha = 0.05$) were considered to be evidence of interaction and were retained in the final model. Interaction terms were interpreted graphically and with contrast statements. Variables were retained in the final multivariable model if they were statistically significant ($\alpha = 0.05$), were part of a significant interaction term, or acted as a confounder.

Model fit was assessed by plotting residuals and BLUP and visually assessing whether the assumptions of homoscedasticity and normality were met. Standard-

ized residuals were examined graphically to look for outliers. Intraclass correlation coefficients (**ICC**) were calculated for each model to determine the amount of total variance in outcome that could be attributed to variation between farms.

Multivariable Mixed Logistic Regression Models. Multivariable mixed logistic regression models were built using adaptive quadrature with the *xtnlogit* command in Stata software, including farm as a random intercept. The outcome variables for these models included treatment for BRD, treatment for other diseases, and mortality.

The model-building process for logistic regression was similar to that of the linear models, with the exception of the methods to calculate ICC, and in assessing the overall fit of the models. For each logistic model, ICC was calculated using the latent variable method. If the variance component was small, and the random effect was not statistically significant ($\alpha = 0.05$), the Akaike information criterion and Bayesian information criterion were used to compare the mixed logistic model with a regular logistic model. The model with the lowest Akaike information criterion and Bayesian information criterion value was selected as the final model. A Hosmer-Lemeshow goodness-of-fit test was performed to assess the overall fit of the models. Pearson and deviance residuals were examined graphically to look for outliers and $\Delta\beta$ was assessed for influence on the coefficients of the models. Residuals were assessed graphically, and in mixed models, BLUP were examined for normality.

Cut-Point Analysis. A range of cut points for Hp concentrations in calves 1 to 8 d of age were evaluated for association with treatment for any disease, and for mortality before 4 mo of age. Cut points within the biological range of Hp were assessed by calculating the sensitivity and specificity and receiver operator characteristic curves were produced. Positive and negative predictive values were evaluated using 2-by-2 tables.

RESULTS

Description of Calves

A total of 1,365 Holstein heifer calves were enrolled from 15 dairy farms. A mean of 91 calves were enrolled per farm (range: 9 to 422). However, not all calves had complete data records, including 134 calves in which Hp results were not available. Almost half of all calving events were unobserved by farm personnel (48.5%). The remaining calves were born from an observed but unassisted calving (12.6%), an easy pull (32.5%), a hard pull (3.8%), malpresentation (2.0%), or surgery (0.6%).

Hp

Concentrations of Hp were obtained from 1,231 calves from 1 to 8 d of age. These Hp values ranged from 0.00 to 1.32 g/L, with a mean ($\pm$ SD) of 0.13 ± 0.13 g/L. The average values of Hp by farm ranged from 0.08 to 0.18 g/L.

As shown graphically in Figure 1, a model investigating the effect of calving difficulty on serum Hp concentration was not statistically significant, even when controlling for the age at first sampling (1 to 8 d). Yet, as the age at first sampling increased, so did the mean serum Hp concentration ($\beta = 0.0094$; 95% CI = 0.0059 to 0.013; $P < 0.001$).

The univariable association between Hp and STP values measured in calves 1 to 8 d of age revealed that calves with higher serum Hp had significantly lower STP concentrations ($\beta = -0.33$; 95% CI = -0.61 to -0.054 ; $P = 0.019$). Furthermore, having a higher serum Hp concentration was significantly associated with increasing the odds of the future treatment for BRD [odds ratio (**OR**) = 2.66; 95% CI = 1.01 to 7.00; $P = 0.048$], treatment for other diseases (**OR** = 12.59; 95% CI = 4.11 to 30.51; $P < 0.001$), and death (**OR** = 8.67; 95% CI = 2.49 to 30.21; $P = 0.001$) at the univariable level.

In a multivariable model, predictor variables associated with increased serum Hp concentrations in the first week of life included having a higher rectal temperature and attitude score (a higher attitude score indicative of a more depressed calf) within the first week of life (Table 1). In addition, a significant interaction was identified between age and nasal score at first sampling with Hp concentration. Serum Hp concentration increased in calves with an increasing nasal score, and the effect was larger when calves were older upon Hp sampling (Figure 2).

For the prediction of both morbidity and mortality, the optimal cut point for serum Hp concentration measured in the first week of life was 0.13 g/L. At this cut point, serum Hp concentration was significantly predictive of treatment for any disease ($P = 0.005$) as well as for mortality ($P = 0.03$) over the study period. For the prediction of disease, the sensitivity and specificity for the test was 27% (95% CI = 22.7 to 31.2%) and 80% (95% CI = 77.2 to 83.0%), respectively, with a positive predictive value of 44% (95% CI = 37.8 to 50.0%) and a negative predictive value of 66% (95% CI = 62.3 to 68.5%). For the prediction of mortality, the sensitivity and specificity for the test were 35% (95% CI = 22.2 to 48.6%) and 78% (95% CI = 75.7 to 80.6%). The positive predictive value was poor (7%; 95% CI = 4.3 to 10.7%) at this cut point, but the negative predictive value was very good (96%; 95% CI = 94.7 to 97.3%).

Figure 1. Mean ($\pm$ SE) haptoglobin concentrations for heifer calves aged 1 to 8 d by level of assistance at birth, based on raw data.

ADG

Average daily gain was calculated for each calf. The mean ($\pm$ SD) ADG of all calves was 0.86 ± 0.21 kg/d. Haptoglobin concentration in the first week of life was not significantly associated with ADG (Table 2). However, ADG was significantly improved in calves that were born from a higher-parity dam, calves that shared airspace with older cattle, and with certain methods of milk feeding. Conversely, being born in the winter, spring, or summer compared with the fall, having a

looser fecal consistency at the third sampling time, and being treated for disease had a negative effect on ADG (Table 2).

Total Health Score

Haptoglobin concentration at the first sampling time was significantly associated with total health score over the study period (Table 3). Other variables in this model that were significantly associated with a higher total health score included being born in the winter

Table 1. Multivariable mixed linear regression model of the predictors of haptoglobin (Hp) at the first sampling time (1 to 8 d of age), including farm as a random intercept (n = 1,096)¹

Variable	Hp (g/L)			
	Coefficient	95% CI		P-value
		Lower limit	Upper limit	
Age at sampling event 1 (d)	0.007	0.003	0.009	0.011
Attitude score at sampling event 1	0.027	0.007	0.047	0.009
Rectal temperature at sampling event 1 (°C)	0.032	0.017	0.048	<0.001
Nasal score at sampling event 1	−0.020	−0.054	0.014	0.26
Interaction				
Nasal score and age at sampling event 1	0.012	0.005	0.018	0.001
Random-effects parameter	95% CI			
	Variance	Lower limit	Upper limit	P-value
Farm level	0.0003	0.00007	0.0015	0.009
Calf level	0.016	0.015	0.017	

¹The intraclass correlation coefficient reveals that 2.0% of the total variance in Hp concentration can be attributed to variation between farms.

Figure 2. Predicted values for haptoglobin (Hp; g/L) in the first week of life as nasal score interacts with days of age at first sampling. All other variables are set to median values. Values based on a mixed linear regression model.

compared with the fall, feeding a lower volume of colostrum, and certain cleaning practices for preweaning housing (Table 3).

Treatment for Disease

The risk of treatment for BRD at least once during the study period was 26.5% in calves <4 mo of age, with individual farm risks ranging from 0 to 79.9%. Haptoglobin concentration in the first week of life did not significantly increase the odds of being treated for BRD (Table 4). Variables that did significantly increase the odds of treatment for BRD at least once during a calf's enrollment on the study included being a twin, navel dipping following birth, having been treated for a disease other than BRD before the second sampling (15 to 21 d), and being fed by an automatic feeder compared with a bucket during the preweaning period (Table 4).

In calves <4 mo of age, the overall risk of treatment for diseases other than BRD was 18.9%. By farm, this risk ranged from 0 to 84.4%. Haptoglobin concentration in the first week of life significantly increased the odds of being treated for diseases other than BRD (Table 5). For every 1 g/L increase in Hp concentration, the odds of being treated for disease increased by 7.6 times. Other variables associated with increasing the odds

of treatment included being a twin and having failure of passive transfer (<5.2 g/dL). Furthermore, being housed in a hutch compared with an indoor individual pen significantly reduced the odds of treatment for disease (Table 5).

Mortality

The risk of mortality in calves <4 mo of age across all farms was 4.4%. By farm, this risk ranged from 0 to 7.7%. Serum Hp concentration at first sampling was significantly associated with mortality in calves <4 mo old (Table 6). Other variables associated with increasing the risk of mortality included failure of passive transfer and treatment for diseases including BRD and diarrhea (Table 6). This model was best fit using a regular logistic model, rather than a mixed model.

DISCUSSION

Trauma at birth is important to identify in newborn calves, as they may be at increased risk of failure of passive transfer due to a delay in the motivation to suckle colostrum or a decrease in colostral immunoglobulin uptake by the intestine (Boyd, 1989; Barrier et al., 2012). If trauma is found to be severe, further intervention may be needed to improve calf vitality, preventing

Table 2. Multivariable mixed linear regression model of the predictors of ADG over the study period, including farm as a random intercept (n = 1,033)¹

Variable	ADG (kg/d)			
	Coefficient	95% CI		P-value
		Lower limit	Upper limit	
Haptoglobin (g/L)	0.047	−0.035	0.13	0.26
Season of birth				
Fall	Ref ²	—	—	—
Spring	−0.059	−0.087	−0.032	<0.001
Summer	−0.072	−0.099	−0.046	<0.001
Winter	−0.11	−0.15	−0.078	<0.001
Dam parity	0.0075	0.00026	0.015	0.042
Shared airspace with older cattle				
No	Ref	—	—	—
Yes	0.12	0.011	0.22	0.03
Fecal score at third sampling ³	−0.038	−0.056	−0.02	<0.001
Treated for any disease at least once during the study				
No	Ref	—	—	—
Yes	−0.044	−0.068	−0.021	<0.001
Mode of milk delivery				
Bucket	Ref	—	—	—
Automatic feeder	0.016	−0.16	0.20	0.86
Bottle	−0.13	−0.25	−0.00071	0.049
Bottle and automatic feeder	0.25	0.11	0.39	<0.001
Bottle and bucket	0.077	−0.024	0.18	0.14
Bucket and automatic feeder	0.052	−0.091	0.19	0.48
Bucket and nipple bar	−0.072	−0.20	0.15	0.78
Nipple bar	0.031	−0.15	0.21	0.74
Random-effects parameter				
Farm level	Variance	Lower limit	Upper limit	P-value
Calf level	0.0035	0.0009	0.014	<0.001
	0.027	0.025	0.029	

¹The intraclass correlation coefficient reveals that 12.0% of the total variance in ADG can be attributed to variation between farms.

²Ref = referent category.

³Third sampling = 36 to 42 d of age.

long-term risks for health and survival (Murray and Leslie, 2013). Identification of a biomarker for calving difficulty may be a more reliable indication of trauma and inflammation than a subjective calving score.

This study is one of few to investigate factors associated with serum Hp levels in newborn dairy calves. Although Hp concentrations were not statistically different by calving difficulty, it is noteworthy that Hp was numerically higher in calves born from a hard pull, malpresentation, and surgery. However, the sample sizes in these groups were much lower than those born from the other assistance categories. This distribution of calving difficulty scores may have resulted in a lack of power to detect a difference in Hp concentrations. In addition, scores for calving difficulty were assigned by different individuals by farm and within farm. Although all individuals received equal training in this scoring technique before calf enrollment, issues may have existed that were associated with interobserver

reliability and misclassification bias. Currently, no validated protocol exists for objective assessment of calving difficulty that can be used in field data collection. Although higher scores generally reflect a greater level of assistance, the actual force applied is not quantified (Meijering, 1984). Thus, it cannot be concluded that all calves born from a hard pull endured a comparable degree of trauma, resulting in a similar inflammatory and acute-phase response.

In agreement with the current study, Alsemgeest et al. (1995) found no association between stress associated with parturition and serum Hp levels in the newborn calf. However, blood samples for Hp detection in that study were collected within 10 min of birth, and hepatic synthesis of Hp may not be fully developed immediately following birth. Orro et al. (2008) reported that over the first 3 wk of a calf's life, serum Hp concentration was highest in the first 3 d. However, it remains unclear whether this increase was due to the

Table 3. Multivariable mixed linear regression model of the predictors of total health score, including farm as a random intercept (n = 1,035)¹

Variable	Total health score			
	Coefficient	95% CI		P-value
		Lower limit	Upper limit	
Haptoglobin (g/L)	1.26	0.16	2.37	0.025
Season of birth				
Fall	Ref ²	—	—	—
Spring	0.22	−0.15	0.60	0.25
Summer	0.20	−0.15	0.56	0.26
Winter	0.76	0.29	1.23	0.001
Total colostrum volume fed (L)	−0.13	−0.24	−0.018	0.023
Prewaning pen cleaned				
Between calves (individual pen)	Ref	—	—	—
Between calves (hutch)	−0.46	−1.60	0.69	0.44
Every 2 to 4 wk (hutch), between groups (pens)	−0.42	−2.14	1.31	0.64
Between calves (hutch), between groups (pen)	−0.41	−1.81	0.98	0.56
Between calves (hutch), 2×/mo (pens)	−2.13	−4.01	−2.50	0.026
Monthly (group pen)	0.61	−1.21	2.42	0.51
Weekly (individual pen)	−2.39	−4.68	−0.11	0.04
95% CI				
Random-effects parameter	Variance	Lower limit	Upper limit	P-value
Farm level	0.57	0.16	1.95	<0.001
Calf level	4.96	4.54	5.41	

¹The intraclass correlation coefficient reveals that 10.2% of the total variance in total health score can be attributed to variation between farms.

²Ref = referent category.

effects of calving or other factors such as disease. It is noteworthy that there were only 13 calves in the Orro et al. (2008) study, 2 of which were born with forceful extraction. Further investigation of serum Hp levels a few days following a difficult calving is warranted.

It is clear that Hp concentration is dependent on the age at blood sampling, which presents a limitation of this study. As calves were sampled from 1 to 8 d of age, multiple confounding factors may have been present, although attempts were made to control for age at sampling in the multivariable models. In addition, with the size and scope of this observational study, the specific cause of elevated Hp concentration in the first week of life could not be determined. Although it is possible that trauma following a difficult calving caused tissue damage and an increase in serum Hp concentration, high Hp concentration could also be due to a bacterial or viral infection at the time of sampling. To understand the long-term health risks associated with high Hp concentration, it is necessary to determine the specific cause of this increase. In future research, efforts should be made to quantify the state of trauma and degree of inflammation associated specifically with calving difficulty or disease.

Based on past research in calves, it is clear that Hp concentration increases following infection. Several

studies observed a significant increase in Hp concentration in response to experimentally induced infection (Conner et al., 1989; Godson et al., 1996; Heegaard et al., 2000) or naturally occurring infection (Angen et al., 2009). It was found that the development of fever and the severity of disease were highly associated with the Hp response to challenge with respiratory viruses and bacterial pneumonia (Godson et al., 1996; Heegaard et al., 2000). Thus, it is not surprising that in the current study, Hp concentration was significantly higher in calves with an elevated rectal temperature and attitude score (more depressed) at the time of sampling. In addition, an interaction existed between nasal score and age at blood sampling for Hp. This finding suggests that the severity of nasal discharge is associated with the Hp response, but is also dependent on the age of the calf. This relationship is consistent with other literature suggesting that the Hp response to infection is delayed compared with other acute-phase proteins (Horadagoda et al., 1994; Heegaard et al., 2000).

The association of Hp concentration with future health in calves has not been previously investigated. However, cows with an Hp concentration of ≥ 1 g/L on d 3 postcalving were 6.7 times more likely to be subsequently diagnosed with mild or severe metritis (Huzzey et al., 2009). The current study supports the idea that

Table 4. Multivariable mixed logistic regression model of the predictors for treatment for bovine respiratory disease (BRD) at least once before 4 mo of age, including farm as a random intercept (n = 1,181)¹

Variable	Treatment for BRD			
	Odds ratio	95% CI		P-value
		Lower limit	Upper limit	
Haptoglobin (g/L)	2.02	0.75	5.47	0.16
Navel dipped				
No	Ref ²	—	—	—
Yes	2.32	1.07	5.06	0.034
Twin				
No	Ref	—	—	—
Yes	2.57	1.03	6.38	0.043
Treated for disease other than BRD before second sampling ³				
No	Ref	—	—	—
Yes	1.57	1.03	6.38	0.043
Mode of milk delivery				
Bucket	Ref	—	—	—
Automatic feeder	22.3	1.51	329.98	0.024
Bottle	0.27	0.018	4.01	0.34
Bottle and automatic feeder	5.19	0.38	71.03	0.22
Bottle and bucket	1.41	0.22	9.19	0.72
Bucket and automatic feeder	6.54	0.44	96.97	0.17
Buck and nipple bar	8.07	0.56	115.98	0.13
Nipple bar	4.99	0.33	75.83	0.25
95% CI				
Random-effects parameter	Variance	Lower limit	Upper limit	P-value
Farm level	1.06	0.32	3.46	<0.001

¹The intraclass correlation coefficient reveals that 24.4% of the total variance in BRD treatment can be attributed to variation between farms.

²Ref = referent category.

³Second sampling = 15 to 21 d of age.

serum Hp concentration shows considerable promise as an indicator of future morbidity and mortality.

Serum Hp concentration in the first week of life was strongly associated with treatment for diseases other than BRD. Diseases such as neonatal diarrhea may predispose calves to BRD (Waltner-Toews et al., 1986a; Van Donkersgoed et al., 1993). This is supported by the current study, as calves that were treated for other diseases before 15 to 21 d of age (second sampling) had increased odds of treatment for BRD. It is possible that disease before the second sampling may be an intervening variable between serum Hp concentration and BRD. In addition, calves may be more likely to have a higher serum Hp concentration in their first week of life if they are susceptible to diseases that occur early in the preweaning period.

The negative association between serum Hp and STP concentrations found in the current study is not supported by previous research. However, it has been well documented that calves with failure of passive transfer are at increased risk of infectious disease and mortality (Wittum and Perino, 1995; Weaver et al., 2000;

Windeyer et al., 2014), which is also in agreement with the results of the current study. In addition, a positive association existed between serum Hp concentration at 1 to 8 d of age and the odds of treatment for disease and mortality. As such, similar to STP concentration, it is possible that serum Hp concentration could be used as a predictor of morbidity and mortality events at the group level. It is also possible that the negative association between Hp and STP concentrations was not through IgG specifically, but through other proteins such as albumin. Other research has found the production of albumin decreases during an acute-phase response (Murata et al., 2004).

In the current study, treatment for BRD, diarrhea, dullness, omphalophlebitis, arthritis, or other diseases increased the risk of mortality in calves <4 mo of age. Diarrhea and respiratory disease alone have been shown to account for 56.5 and 22.5%, respectively, of all preweaned heifer mortality (USDA, 2007). In agreement, Waltner-Toews et al. (1986b) found that heifers treated for pneumonia were 2.5 times more likely to die after 90 d of age than heifers not treated. Furthermore, other

Table 5. Multivariable mixed logistic regression model of the predictors for treatment for diseases other than bovine respiratory disease at least once before 4 mo of age, including farm as a random intercept (n = 1,181)¹

Variable	Treatment for other diseases			
	Odds ratio	95% CI		P-value
		Lower limit	Upper limit	
Haptoglobin (g/L)	7.57	2.83	20.21	<0.001
Twin				
No	Ref ²	—	—	—
Yes	2.64	1.21	5.77	0.014
Passive transfer				
Pass (>5.4 g/dL)	Ref	—	—	—
Intermediate (5.2–5.4 g/dL)	1.50	0.93	2.42	0.099
Fail (<5.2 g/dL)	1.84	1.19	2.83	0.006
Type of preweaning pen				
Individual pen	Ref	—	—	—
Hutch	0.25	0.11	0.59	0.001
Hutch and then individual pen	1.97	0.76	5.09	0.16
Hutch and then small group	0.96	0.35	2.60	0.94
Hutch and then medium group	1.31	0.55	3.11	0.54
Small group	0.31	0.076	1.24	0.098
Random-effects parameter	95% CI			
	Variance	Lower limit	Upper limit	P-value
Farm Level	0.15	0.027	0.82	0.037

¹The intraclass correlation coefficient reveals that 4.3% of the total variance in disease treatment can be attributed to variation between farms.

²Ref = referent category.

studies have indicated that calf dullness was associated with an increased risk of mortality (Curtis et al., 1989; Warnick et al., 1997).

The relationship between serum Hp concentration and calf mortality has not been previously investigated.

In the current study, serum Hp concentration was associated with mortality, both directly and indirectly through its effect on the risk of treatment for disease. Other research has proposed an association between dystocia and an increased risk of mortality. The risk

Table 6. Multivariable logistic model of the odds of mortality in calves < 4 mo of age (n = 1202)

Variable	Mortality			
	Odds ratio	95% CI		P-value
		Lower limit	Upper limit	
Haptoglobin (g/L)	4.29	1.07	17.18	0.04
Passive transfer				
Pass (>5.4 g/dL)	Ref ¹	—	—	—
Intermediate (5.2–5.4 g/dL)	0.43	0.13	1.47	0.18
Fail (<5.2 g/dL)	2.22	1.2	4.11	0.01
Treated for BRD once or more during study period ²				
No	Ref	—	—	—
Yes	2.77	1.56	4.92	0.001
Treated for scours once or more during study period				
No	Ref	—	—	—
Yes	3.26	1.72	6.18	<0.001
Treated for any other disease once or more during the study period				
No	Ref	—	—	—
Yes	2.06	1	4.22	0.049

¹Ref = referent category.

²BRD = bovine respiratory disease.

of mortality within the first week of life increased in calves affected by dystocia (Schuijt and Taverne, 1994). If the Hp levels in the current study correlate with the inflammatory response at calving, the increased risk of mortality may be partly caused by the effects of dystocia.

From the results of this study, good evidence exists to suggest that serum Hp concentration in the first week of life is associated with the future risk of disease and mortality. Serum Hp concentrations of >0.13 g/L in calves during the first week of life has potential utility for identification of groups of calves that have increased risk of disease and death. Yet, using this cut point would not be optimal for the identification of individual calves at increased risk of disease and death, as the sensitivity and specificity of this test were low. In other words, these results do not support the use of serum Hp concentrations alone as an individual animal test. Similar to STP concentration for the assessment of failure of transfer of passive immunity, the utility of measurement of Hp concentration in the first week of life would be best applied at the group level for overall assessment of calving management practices on future health and mortality.

CONCLUSIONS

Serum Hp concentration at 1 to 8 d of age was not significantly higher in calves born from a difficult calving compared with those born from an easy pull or unassisted at calving. However, calves that were unhealthy at 1 to 8 d of age were more likely to have higher serum Hp concentrations. Furthermore, serum Hp concentration in the first week of life was significantly associated with the future risk of treatment for diseases other than BRD, as well as for mortality. Measuring serum Hp concentration in the first week of life was not an ideal test for the correct classification of individual calves that will develop disease or die within their first 4 mo, due to low test sensitivity. Monitoring serum Hp concentration in the first week of life shows potential at the group level for the overall assessment of calving management practices on future health and mortality.

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