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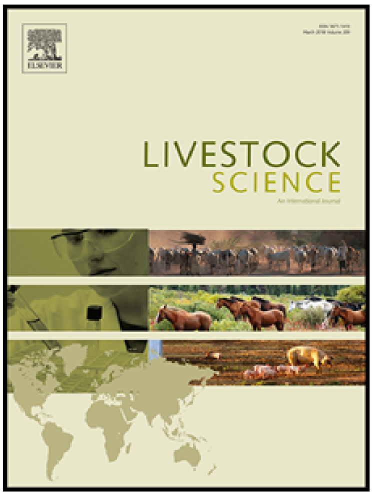

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Cut-off values for predictors associated with outcome in dairy calves suffering from neonatal calf diarrhea. A retrospective study of 605 cases

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Highlights

- Clinical data of 605 diarrheic calves were evaluated to identify predictors associated with outcome
- Calves with total serum protein (STP) >50 g/L had greater odds of surviving
- Disease severity did not influence the relationship between STP concentration and outcome
- Based on dehydration degree STP cut-off value ranged between 45 and 55 g/L
- None of the markers used had great predictive ability to assess mortality risk

Abstract

The considerable impact of neonatal calf diarrhea (NCD) on animal health and productivity prompted us to review the clinical and laboratory data in diarrheic calves presented to a referral institution.

This study aimed to identify the major predictors associated with outcome in affected calves undergoing a standard therapy protocol and to evaluate the diagnostic performance of significant predictors for identifying the corresponding optimal cut-off values associated with outcome.

Signalment, clinical signs, serum total protein (STP) concentration measured with an optical refractometer, venous blood gas analysis, treatment, and outcome were reviewed in 605 Holstein Friesian calves with uncomplicated NCD over 12 years. Calves were treated according to the severity of the clinical status with 3 standard fluid therapy regimens.

After therapy, 414 calves were discharged in a healthy state, and 191 died. A multivariable logistic regression model indicated that survival was associated with STP concentration (odds ratio: 2.74; 95% confidence interval: 2.10-3.56; $P < 0.001$), rectal temperature (odds ratio: 1.22; 95% confidence interval: 1.03-1.44; $P = 0.024$) and blood pH (odds ratio: 7.49; 95% confidence interval: 1.33-42.18; $P < 0.022$). Receiver operating characteristic analysis showed that the optimal cut-off value of STP for predicting positive outcome was >50 g/L, associated with a sensitivity (Se) of 71.2%, a specificity (Sp) of 71.3%, a positive predictive value (PPV) of 84.5%, and a negative predictive value (NPV) of 53.2%. Optimal cut-off values for predicting positive outcome were rectal temperature >38.3°C (Se=61.6%, Sp=55.5%, PPV=74.9%, NPV=40.1%) and blood pH >7.12 (Se=68.7%, Sp=42.4%, PPV=71.4%, NPV=39.3%), despite the poor accuracy of these predictors according to the area under the curve (AUC). When association between predictors and outcome was analyzed split according to treatment regimens, the only predictor associated with outcome was STP concentration. Collectively, these findings suggest that STP is effective as a positive prognostic factor for diarrheic calves, with an STP concentration >50 g/L associated with a higher probability of survival. Additionally, our findings suggest that high STP may influence calf health during NCD.

Key words

Calves; Neonatal calf diarrhea; Outcome; Refractometer; Serum total protein

Introduction

Neonatal calf diarrhea (NCD) is the most common disease in pre-weaned dairy calves. It causes considerable economic losses worldwide due to mortality, treatment costs and poor growth (USDA, 2012; Torsein et al., 2011). The mortality risk due to diarrhea ranges from 5% to 24% in field conditions (Fecteau et al., 1997; Windeyer et al., 2014), and 20% to 30% in hospital conditions (Lorenz, 2004; Boccardo et al, 2017; Trefz et al., 2017), where more severe cases are usually treated. Previous studies have reported a consistent relationship between the renal and pulmonary function and evidence of septicemia with mortality in NCD-affected calves (Seifi et al., 2006; Bonelli et al., 2018; Tsukano et al., 2018). During NCD episodes, prognostic information needs to be obtained as soon as possible, to guide the economic evaluation and sustainability of treatments.

Trefz et al. (2017) showed that clinical manifestations, such as central nervous system symptoms, cachexia, and abdominal emergencies, were easy to detect and were strongly associated with mortality risk. On the other hand, laboratory values were of limited use for predicting the outcome in calves with NCD, although low serum total protein (STP) concentration, measured by an optical refractometer, has been associated with case fatality in hospitalized diarrheic calves treated with a standard protocol (Boccardo et al., 2017).

There are currently no data on a cut-off value for the easiest-to-obtain clinical and laboratory findings associated with prognosis in calves affected by NCD. Having prognostic cut-off values may help practitioners during NCD episodes to distinguish between calves with a positive prognosis and calves at a high risk of mortality. To address this knowledge gap and to identify the major risk factors for outcome in diarrheic calves, we examined clinical data, the STP concentration measured by a serum refractometer, and venous blood gas analysis. The aim was to determine the optimal cut-off values found to have a significant predictive value in the outcome of calves with NCD.

Materials and methods

Case selection and clinical procedures

The study sample consisted of client-owned dairy calves admitted to the Veterinary Teaching Hospital of the University of Milan with a clinical diagnosis of NCD on initial examination. Calves were retrospectively recruited from patients admitted between 1 January 2007 and 31 December 2019. The clinical diagnosis of NCD was based on the presence of watery feces at admission in calves up to 21 days of life. Because of regional preferences, dairy calves admitted to our clinic were mainly Holstein Friesian (> 90%) and therefore only these calves were considered for the study. To rule out other pathological conditions that would bias the results (Trefz et al., 2017), other concurrent diseases at the time of hospitalization represented an exclusion criterion.

Information retrieved from medical records included clinical and laboratory findings from the initial examination, therapy, and outcome. Clinical procedures and treatment followed a standardized protocol to manage NCD: on admission, diarrheic calves were submitted to a complete clinical examination. Bodyweight (kg), age (d), and rectal temperature (°C) were recorded for each animal. Clinical scores were used for the evaluation of vigor, dehydration and suckle reflex, based on previous investigations (Boccardo et al, 2017; Boccardo et al, 2019), as briefly summarized in Table 1.

A blood sample was collected in a 9-mL tube without anticoagulant from the jugular vein and used to determine STP concentration. Samples were allowed to clot and then centrifuged at 20 °C for 10 min at 900 g. The STP concentration was determined with a hand refractometer (mod. MR514ATC, Milwaukee srl, Gallarate, Italy). After centrifugation, the serum was aspirated into a soft plastic Pasteur pipette, a drop of serum was then used for the analysis. A blood sample for venous blood-gas analysis was also anaerobically collected from the jugular vein into a disposable heparinized 2.5 mL syringe. Blood pH, bicarbonate (HCO_3^-), the partial pressure of carbon dioxide (pCO_2), base excess (BE), blood sodium (Na^+), chlorine (Cl^-), potassium (K^+), anion gap (AG), packed cell volume (PCV), and hemoglobin (Hb) were immediately determined using a blood gas analyzer (AVL Opti CCA, Diamond Diagnostic, Holliston, USA).

All calves included in the study were managed according to standard protocols for the treatment of NCD and in compliance with the professional ethics for veterinarians (FNOVI, 2019) and the standards for the protection of calves (European Commission, 2008).

Treatment, housing, and outcome

The standard therapy followed guidelines based on the results of a previous retrospective analysis (Boccardo et al., 2017; Boccardo et al., 2019). Briefly, on admission, diarrheic calves with strong suckle reflex, vigor score ≥ 4, dehydration score ≤ 1, and base excess up to -8 mmol/L received 1 L of oral rehydration solution (ORS) containing 4 g sodium chloride, 20 g dextrose, 3 g potassium bicarbonate, and 3 g sodium propionate. One L of ORS was additionally administered three times between milk feedings during the first 24 h after admission (treatment regimen 1).

Calves with a vigor score < 4 or dehydration score > 1 or base excess < -8 mmol/L received a constant drip infusion consisting of 5 L of isotonic saline spiked with 8.4% sodium bicarbonate ($NaHCO_3$) (calculated according to Lorenz and Vogt, 2006) at constant drip infusion (40 mL/kg/h) according to the following formula: bicarbonate requirements (g) = body weight (kg) × B.E. (mmol/L) × 0.6 (L/ kg) × 0.084 (g/mmol). Calves with a history of malnutrition or lack of milk intake for more than 12 hours received 400 mL of a 50% glucose solution added to the saline solution (treatment regimen 2).

Calves with a dehydration degree ≥ 2 received an additional 5 L bag of isotonic saline at a slow infusion rate (10 mL/kg/h) (treatment regimen 3).

Calves of treatment 1 that had not finished drinking the ORS at the time of admission or between milk feedings, received a constant drip infusion consisting of 5 L of isotonic saline spiked with 8.4% $NaHCO_3$ at a slow infusion rate (10 mL/kg/h). Calves that showed deterioration (or failed improvement) of hydration, vigor and suckle reflex scores, 24 h after therapy, or deterioration after an initial improvement, were subjected to an additional venous blood gas analysis. These unresponsive calves were then treated with further IV fluid therapy based on their current acid-base and dehydration status. At the end of the fluid therapy, 2 L milk replacer was offered three times a day.

Calves with at least one of the systemic signs of illness at admission or during hospitalization (e.g. 24 h of inappetence, dehydration score of 2 or more, lethargy, pyrexia, or bloody diarrhea) received amoxicillin and clavulanic acid (Synulox, Zoetis Italia, Roma, Italy) subcutaneously at a dose of 10 + 2.5 mg/kg for 5 days. Hypothermic (<38.5 °C) calves were placed under an infrared lamp. On the day of hospitalization, flunixin meglumine (Alivios, Fatro, Ozzano dell'Emilia, Italy) at a dose of 2.2 mg/kg IV, vitamin E and selenium (Selevit, Fatro, Ozzano dell'Emilia, Italy) and group B vitamins (Dodicile, Fatro, Ozzano dell'Emilia, Italy) were administered parenterally in all calves.

During hospitalization, calves were housed in individual calf brick pens (1.8 m × 1.2 m) in an indoor 30-place stall with a controlled temperature of 18 °C. The pens were bedded with straw and cleaned every day. Freshwater, hay and calf starter were offered *ad libitum*. Calves were monitored twice daily with complete clinical examinations. Surviving calves were discharged from the hospital in a healthy state, i.e. normal hydration and vigor scores, good appetite, and normal consistency of the feces for at least two days.

Data analysis

For all statistical analyses, SPSS 26.0 for Mac (IBM, Armonk, USA) was used. Descriptive statistics were performed, and continuous variables were expressed as the mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages with 95% confidence intervals. Continuous variables included in the statistical analysis were age, body weight, rectal temperature, STP concentration, and venous blood gas findings. The categorical variables included were sex, dehydration score, vigor score, suckle reflex, fluid treatment regimens, and eventual treatment with antibiotics. Statistical analysis was performed using only laboratory and clinical data collected at admission. To identify which of these variables were correlated with outcome, a multivariable logistic regression model was performed. In the univariable model, we included all variables listed above and 2-way interaction term. Variables or interactions identified as potentially useful predictors (*P* <0.1) were then insert in the final model using forward stepwise regression to estimate the probability of surviving or dying. Only those variables or interactions that presented a *P* <0.05 on the final regression model were considered to be correlated with outcome. The linearity between the continuous predictors and outcome was assessed visually by plotting the predictors against the log odds of survival. To quantify the validity of the regression model, r-squared coefficients were checked.

To assess if treatment regimens could influence the association between predictors and outcome, data were grouped according to treatment regimens, and the same multivariable logistic regression model was then applied to the grouped data.

Based on the results of multivariable logistic regression analysis, a receiver operating characteristic (ROC) curve was performed for each of the continuous predictors that were significantly associated with the outcome. The ROC curves were built to establish the optimal cut-off value associated with positive outcome (survival) in diarrheic dairy calves. The optimal cut-off point was chosen using the Youden index, where sensitivity (Se) and specificity (Sp) are maximized, and equal weight is given to false-positive and false-negative results. The calculated cut-off values were used to calculate Se, Sp, positive predictive value (PPV), and negative predicted value (NPV). In addition, the area under the curve (AUC) and its 95% confidence intervals (CI) were calculated and used as an indicator of the accuracy of the parameters. Interpretation of AUC was based on the following scoring system: 1.0 perfect test, 0.99–0.90 excellent test, 0.89–0.80 good test, 0.79–0.70 fair test, 0.69–0.51 poor test, and 0.50 or lower fail (Hanley and McNeil, 1982).

Based on our early observation that STP concentration was associated with neonatal mortality in calves with NCD (Boccardo et al. 2017) and based on the observation that STP concentration may change appreciably with dehydration (Thornton et al., 1972), other cut-off values for STP were calculated with ROC curves using data split for/grouped according to dehydration scores. These cut-off values were then used to calculate Se, Sp, PPV, NPV and AUC, as previously reported/described above.

Results

From a total of 5007 cattle admissions over the considered period, a dataset of 781 diarrheic Holstein Friesian calves was initially reviewed. The medical records of 605 calves (528 females and 7 males) met the criteria for inclusion in the study. Analysis of data retrieved from the medical records is summarized in Tables 2 and 3.

The 605 NCD calves were assigned to standard treatments based on clinical and acid-base status: oral rehydration (treatment regimen 1, n = 82), 5 L of isotonic saline spiked with 8.4% sodium bicarbonate (treatment regimen 2, n = 147), additional 5 L bags of isotonic saline at a slow infusion rate (treatment regimen 3, n = 376). Glucose was added to the infusion fluids in 545 cases. Antimicrobial therapy was performed in 563 cases. The infrared lamp was used in 302 cases. The report of clinical improvement or description of further treatments in unresponsive calves or calves with deterioration in their general condition after an initial improvement is summarized in Table 4.

Overall, the number of calves that survived or died was 414 (68.4%) and 191 (31.6%), respectively. Of the 191 deceased calves, 62 calves died spontaneously, whereas 129 calves were euthanized for ethical reasons in the case of no response to treatment for ongoing anorexia, depression, severe weakness or cachexia (74 calves), development of meningitis (24 calves), polyarthritis (17 calves), unresponsive pneumonia (10 calves), and peritonitis (4 calves).

The results of the univariable logistic regression revealed an association between positive outcome and STP concentration (*P* <0.001; odds ratio 2,74; CI 95% 2.10–3.56), rectal temperature (*P* =0.022; odds ratio 1.22; CI 95% 1.03–1.44), blood pH (*P* =0.021; odds ratio 7.49; CI 95% 1.33–42.18). Additionally, the only 2-way interaction associated with positive outcome was the one between STP concentration and rectal temperature (*P* <0.001; odds ratio 1.03; CI 95% 1.02–1.03) as well as that one between age and vigour score (*P* =0.010; odds ratio 0.88; CI 95% 0.81–0.94).

When these variables were included in the final model, only a STP concentration (P <0.001; odds ratio 2.74; CI 95% 2.10–3.56), rectal temperature (P =0.024; odds ratio 1.22; CI 95% 1.03–1.44) and blood pH (P =0.022; odds ratio 7.49; CI 95% 1.33–42.18) were predictive for a positive outcome. The linearity between STP concentration, rectal temperature, blood pH and outcome was confirmed visually by plotting the predictors against the log odds of survival.

When data were split according to treatment regimes, a positive association was identified between STP and odds of surviving consistent across all three treatment groups; a positive association was found between rectal temperature and survival in treatment regime 2 (Table 5).

The ROC curves for STP concentration, rectal temperature, and blood pH are shown in Figure 1. The optimum cut-off point for STP in predicting survival was >50 g/L. The analysis of AUC showed that STP concentration was a fair test to predict outcome (AUC=0.76; CI 95% 0.72–0.80). The optimum cut-off point for rectal temperature in predicting survival was >38.3 °C. The analysis of AUC showed that rectal temperature was a poor test to predict outcome (AUC=0.60; CI 95% 0.55–0.65). The optimum cut-off point for blood pH in predicting survival was >7.12. The analysis of AUC showed that blood pH was a poor test to predict outcome (AUC=0.55; CI 95% 0.49–0.61). The results of Se, Sp, PPV, and NPV for each of the aforementioned predictors are reported in Table 6.

The ROC curves for STP concentration, performed with data split according to dehydration scores, are shown in Figure 2. The optimum cut-off points in predicting survival increased with a rising degree of dehydration scores (dehydration score 0: >45 g/L; dehydration score 1: >50 g/L; dehydration score 2: >50 g/L; dehydration score 3: >55 g/L). For each dehydration score, the analysis of AUC showed that STP concentration was a fair test to predict outcome (dehydration score 0: AUC=0.78; CI 95% 0.64–0.92, dehydration score 1: AUC=0.74; CI 95% 0.65–0.83, dehydration score 2: AUC=0.78; CI 95% 0.72–0.84, dehydration score 3: AUC=0.73; CI 95% 0.64–0.83). The results of Se, Sp, PPV, and NPV of STP concentration corresponding to each dehydration score are reported in Table 7.

Discussion

The current study demonstrated that greater STP concentrations are associate with increased odds of surviving in calves affected by NCD (OR = 2.74). Our cut-off of >50 g/L predicted a positive outcome for diarrheic dairy calves with a satisfactory PPV (ranging from 81.2% to 87.3%), while the NPV (49.1% to 57.8%) underlines the poor ability of STP in predicting a negative outcome. These results suggest that although greater STP concentrations represent an important protective factor in diarrheic calves treated with standard therapy, the mortality rate is a multifactorial issue that is often not necessarily related to a reduction in STP concentration. Indeed, none of the clinical and laboratory markers used in this study showed predictive ability in assessing mortality risk.

The protective effect of a high STP concentration could be due to the effects of passive immunity. A considerable amount of literature provided information on the refractometric measurement of STP as an indirect method to evaluate the consistency of the passive transfer of immunity in healthy calves. This method is inexpensive and easy to perform in field conditions, correlates highly with results obtained by the biuret method (Vandeputte et al., 2011), correlates highly with serum IgG concentration (Wilm et al., 2018), and is widely used to estimate morbidity and mortality (Tyler et al., 1999; Windeyer et al., 2014; Todd et al., 2018). These studies effectively assessed how passive immunity affects the calf's health. The STP concentration is thus commonly used to evaluate the inadequate transfer of passive immunity in healthy calves and may be correlated to the serum immunoglobulin G (IgG) up to nine days of age (Wilm et al., 2018). The results of our retrospective investigation could be explained by the fact that calves with greater STP concentrations might have had a higher transfer of passive immunity and therefore might have a better response to the therapy. Although there may be a positive interference of the passive immunity in surviving calves, the relationship between STP concentrations and passive immunity in diarrheic calves is still an open issue. In fact, the clinicopathological abnormalities related to the disease may alter the relationship between serum IgG concentration and other blood solids due to dehydration or an increase in inflammatory proteins (Fecteau et al., 2013; Buczinski et al., 2018). Moreover, NCD occurs in calves during the first month of life; however, the relationship between STP and passive immunity decreases over time, thus reducing the diagnostic accuracy in older animals (Quigley, 2001; Wilm et al., 2018). Other possible explanations therefore need to be considered.

Recent evidence has shown that NCD is associated with a marked reduction in STP concentration in affected calves (Rocha et al., 2016) due to the reduction in the gamma-globulin fraction that is transferred into the intestinal lumen to counteract the infection (Athanasiou et al., 2019). It could, therefore, be argued that diarrheic calves with high levels of STP concentration may have a lower degree of intestinal migration of blood solids, probably associated with a lower level of enteric infection or due to a non-complicated infection that causes only a light enteric disorder (Kelling et al., 2002).

Another possible explanation for the protective effect of STP is that calves with a high STP concentration probably continued to feed. Calves fed with high levels of dietary crude protein and high levels of milk replacer showed a higher STP concentration than calves fed with a low milk volume and low protein content; the decrease in STP concentration can therefore be considered a consequence of malnutrition (Bartlett et al., 2006). During episodes of NCD, calves may suffer from a severe deterioration in the suckle reflex, with a decreased ability to stand and a lack of interest in feeding, leading to prolonged malnutrition which often persists for long periods before the first clinical examination. Moreover, although contraindicated, many farmers suspend milk feeding in diarrheic calves for many days (causing cachexia) because of a traditional myth that milk feeding during enteritis worsens the course of the disease (Trefz et al., 2017). Malnutrition causes a reduced immune response (Singh et al., 2013), general weakness, and inability to suckle, which were the main causes of euthanasia in unresponsive calves both in this study and in others (Trefz et al., 2017). Because most of the surviving calves in this study had a higher concentration of STP than the non-survivors, we can infer that maintaining proper milk intake in diarrheic calves or treating them before malnutrition becomes critical, can affect the health of the calves and thus improve the survival rate.

According to the applied logistic regression model, treatment regimens did not influence the relationship between STP concentration and outcome. This finding suggests that STP concentration is positively related to the odds of surviving in diarrheic calves, irrespective of the severity of the disease. A comparison of our findings with those of other studies (Lorenz, 2004; Trefz et al., 2017; Boccardo et al., 2017; Trefz et al., 2015) confirms that both laboratory values and pathognomonic NCD signs taken individually are of limited value for predicting outcome in calves with NCD because most of these conditions can be easily managed with fluid therapy. Clinical presentation of the diarrhoeic calves is related to the increase of blood D-lactate concentration (Lorenz, 2004). Previous studies reported that calves with elevated D-lactate concentrations did not need additional specific therapy, as D-lactate concentrations regularly fell following the correction of acidosis and restitution of body fluid volume (Müller et al., 2012). Similarly, affected calves can recover from metabolic acidosis with an alkalizing solution containing a highly effective strong ion difference (i.e. sodium bicarbonate) (Müller et al., 2012).

In addition to STP concentration, we found an association with outcome for blood pH and rectal temperature. The poor accuracy of the blood pH can be explained by the fact that, although metabolic acidosis can have profound negative effects (Randhawa et al., 1980; Kasari, 1999; Cambier et al., 2001), only a few cases persist irrespectively of medical treatment, thus resulting in severe underlying clinical conditions that are probably more related to the therapeutic failure than blood pH in itself (Trefz at al., 2017). Similarly, rectal temperature can be easily managed by both restorations of fluid balance and the use of an infrared lamp. Specifically, these observations might explain the poor accuracy of this predictor in our study. Although high STP concentration and normothermia, take individually, were associated with increased odds of surviving in calves affected by NCD, the interaction between these two parameters and outcome was not identified as a significant predictor during the final regression model. This result can be explained by the fact that high STP concentration and normal rectal temperature were independent in this study.

Dehydration degree did not influence the relationship between STP concentration and outcome, STP cut-off values were higher in diarrheic calves with severe dehydration degree than in those non-dehydrated or with a lower dehydration status. This result was expected and suggests that STP was influenced by the degree of dehydration within our study sample, underlining the importance of integrating STP concentration with the degree of dehydration to obtain more relevant prognostic data.

The main limitation of this study was the fact that this retrospective analysis was carried out on calves in a hospital setting where the admission usually concerns more severe cases or cases where therapy in the field had no effect. Such pretreatment activities are not reported in this retrospective study; therefore, we cannot rule out that they had some effect on the outcome, especially if the milk feeding of the diarrheic calves had been suspended for many days. Furthermore, although case-control studies (survived vs died) are an efficient method for investigating outcomes, there are important limitations inherent to this design (Melamed and Robinson, 2019). In this case, the different management practices of calves before to admission and the different levels of exposure to pathogens could create a potential bias on our results. Lastly, although our mortality data are consistent with those of other studies, PPV depends on the population tested and is influenced by the prevalence of the investigated data. In a population with a low prevalence of the disease, the expected decrease of PPV could lead to an increase in misclassification errors. However, the concentration of STP has shown a strong relevance compared to other markers and, in our opinion, could have good results even in field conditions where the risk of mortality is usually lower. Further research should be performed under field conditions to assess the correlation between STP and outcome in a lower mortality scenario.

We believe that our results might have some clinical applications. Although the underlying mechanisms remain unclear, we found that simply evaluating STP concentration measured by an optical refractometer is an important indication for a positive outcome in diarrheic calves undergoing a standard treatment protocol. Our data showed that calves with STP >50 g/L have a 2.74 times higher probability of survival than calves with a lower STP concentration, disregarding of the severity of their clinical status. In addition to the specific clinical complications that are still negative prognostic factors (Trefz et al., 2017), this finding could be used to help practitioners to collect accurate prognostic information from useful, inexpensive, and easy-to-obtain parameters.

Conclusions

The results of this retrospective study indicate that, at admission, STP concentration is the major predictor associated with outcome in the NCD affected calves investigated. ROC analysis showed that the optimal cut-off value of STP concentration for predicting positive outcomes was >50 g/L, associated with an Se of 71.2%, a Sp of 71.3%, a PPV of 84.5%, and a NPV of 53.2%. Although the degree of dehydration did not affect the association between STP concentration and outcome, it should be taken into account to better identify the most accurate cut-off value. We believe that the reported findings could be useful for practitioners to classify diarrheic calves with a higher success rate of therapy. Additionally, the results of this study indicate that episodes of NCD in animals with adequate STP concentration, usually have a positive outcome also in calves with severe clinical and laboratory abnormalities.

Author statement

Antonio Boccardo: conceptualization, writing-original draft preparation, writing - review & editing. **Giulia Sala**: data curation, formal analysis, software, writing - review & editing. **Vincenzo Ferrulli**: data curation, writing - review & editing. **Davide Pravettoni**: Supervision, writing - review & editing.

Conflict of interest

There are no conflicts of interest including financial, personal or other relationships with other people or organizations.

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Table 1. The clinical score used to evaluate the vigor, degree of dehydration and strength of the suckle reflex in 605 diarrheic Holstein Friesian calves.

Score	Clinical signs
Vigor	
5	Standing securely without assistance, curious, alert.
4	Standing up after encouragement, weak, "sad calf".
3	Sternal recumbency, standing after lifting, "drunken gait'', unstable posture.
2	Permanent sternal/costal recumbency.
1	Lateral recumbency, sometimes comatose.
Dehydration	
0	Normal hydration, upper eyelid skin tent <2 s.
1	Moderate dehydration, eyeball slightly sunken (1-2 mm), and upper eyelid skin tent >2 s but <4 s (estimated loss of BW= 3-5%).
2	Obvious dehydration, sunken eyes (3-4 mm), dry nose, upper eyelid skin tent >5 s (estimated loss of BW= 6-8%).
3	Severely sunken eyes with an easily perceptible distance between the eyeball and the eyelid (≥5 mm), cold ears, legs and oral cavity, dry mouth and nose, upper eyelid skin tent persists (estimated loss of BW= ≥9%)
Suckle reflex	
0	Strong
1	Weak
2	Absent or chewing movements

Table 2. Mean ± standard deviation for the considered continuous variables at admission in 605 survived and deceased diarrheic Holstein Friesian calves.

Variables	Survived calves (*n*= 414)	Deceased calves (*n*= 191)
Age (d)	8.7 ± 5.4	10 ± 5.1
Body weight (kg)	41.7 ± 5.5	40.6 ± 5.6
Rectal temperature (°C)	38.4 ± 1.3	37.7 ± 2.0
Serum total protein concentration (g/L)	5.9 ± 1.3	4.7 ± 1.2
pH	7.2 ± 0.1	7.1 ± 0.2
Partial pressure of carbon dioxide (mmHg)	44.0 ± 11.9	44.6 ± 12.1
Blood bicarbonate (mmol/L)	17.8 ± 8.2	17.2 ± 8.4
Base excess (mmol/L)	-9.8 ± 9.2	-10.9 ± 10.5
Blood sodium (mmol/L)	132.9 ± 13.3	131.9 ± 14.8
Blood potassium (mmol/L)	5.7 ± 1.7	5.6 ± 1.7
Blood chloride (mmol/L)	103.6 ± 13.1	103.8 ± 14.6
Anion gap (mmol/L)	16.8 ± 6.0	16.1 ± 5.9
Packed cell volume (%)	33.8 ± 8.1	33.2 ± 9.0
Hemoglobin (g/dL)	11.2 ± 4.1	11.0 ± 3.0

Table 3. Frequencies and percentages of the categorical variables at admission for surviving and deceased calves.

Variables	Score	Survived calves (*n*= 414)	Deceased calves (*n*= 191)
Dehydration score	0	30 (7.2%)	19 (9.9%)
	1	135 (32.6%)	45 (23.6%)
	2	188 (45.4%)	82 (42.9%)
	3	61 (14.7%)	45 (23.6%)
Vigor score	1	36 (8.7%)	46 (24.1%)
	2	74 (17.9%)	47 (24.6%)
	3	120 (29.0%)	38 (19.9%)
	4	151 (36.5%)	47 (24.6%)
	5	33 (8.0%)	13 (6.8%)
Suckling reflex	0	194 (46.6%)	73 38%)
	1	152 (36.7%)	59 (31%)
	2	66 (16.4%)	59 (31%)
Treatment regimen	1	60 (14.5%)	22 (11.5%)
	2	105 (25.4%)	42 (22.0%)
	3	249 (60.1%)	127 (66.5%)
Antibiotic treatment	Yes	384 (93%)	179 (93.7%)
	No	30 (7%)	12 (6.3%)

Dehydration score. 0 = normal hydration, upper eyelid skin tent <2 seconds; 1= moderate dehydration, eyeball slightly sunken (1–2 mm), and upper eyelid skin tent >2 but <4 seconds (estimated loss of body mass 3–5%); 2 = obvious dehydration, sunken eyes (3–4 mm), dry nose, upper eyelid skin tent >5 seconds (estimated loss of body mass 6–8%); 3 = severe sunken eyes with an easily perceptible distance between the eyeball and the eyelid (≥5 mm), cold ears, legs and oral cavity, dry mouth and nose, upper eyelid skin tent persists (estimated loss of body mass ≥ 9%).

Vigour score. 5 = standing securely without assistance, curious, alert; 4 = standing up after encouragement, weak, 'sad calf';3= sternal recumbency, standing after lifting, 'drunken gait', insecure posture; 2 = Permanent sternal/costal recumbency; 1= lateral recumbency, sometimes comatose.

Suckle reflex. 0 = strong; 1 = weak; 2 = absent or chewing movements.

Treatment regimen. 1 = oral rehydration; 2 = parenteral rehydration with 5 L of isotonic saline spiked with 8.4% $NaHCO_3$ at constant drip infusion (40 mL/kg/h); 3 = parenteral rehydration with additional 5 L of isotonic saline at slow infusion rate (10 mL/kg/h).

Table 4. Results obtained in 605 diarrheic Holstein Friesian calves after a standard treatment protocol with oral rehydration solution (ORS) or isotonic saline with 8.4% sodium bicarbonate ($NaHCO_3$) based on clinical and acid–base status.

Therapeutic protocol	ORS (*n* = 82 calves)[a]	5 L isotonic saline + 8.4% $NaHCO_3$ (*n* = 147 calves)[b]	10 L isotonic saline + 8.4% $NaHCO_3$ (*n* = 376 calves)[c]
Recovery[1] after first fluid/ORS protocol	51/82 (s)	78/147 (s)	174/376 (s)
Spontaneous death during/after first fluid protocol or euthanasia[2] due to severe concurrent diseases or massive deterioration	15/82 (d)	25(147 (d)	69/376 (d)
Calves that recovered after more than one fluid therapy	9/82 (s)	27/147 (s)	75/376 (s)
Spontaneous death during/after secondary fluid protocol or euthanasia due to severe concurrent diseases or massive deterioration	7/82 (d)	17/147 (d)	58/376 (d)

s, surviving calves (n = 414); d, dead calves (n = 191). [1]Calves that after the therapy had a good appetite and drank the water independently, were not dehydrated, and showed a constant improvement in the vigor score even with feces still liquid. [2]Unresponsive calves without clinical improvement 12-24 hours after therapy or with a deterioration of the general condition after primary improvement, and subsequently euthanized due to massive deterioration of their general condition (anorexic/non-responsive/non-dehydrated calves without acid-base disturbances not treated further fluids).

a Diarrheic calves with strong suckle reflex, vigor score ≥ 4, dehydration score ≤1, base excess up to -8 mmol/L received on admission 1 L of ORS containing 4 g sodium chloride, 20 g dextrose, 3 g potassium bicarbonate and 3 g sodium propionate. One liter of ORS was additionally administered three times between milk feedings during the first 24 h after admission.

b Calves with vigor score < 4 or dehydration score > 1 or base excess < -8 mmol/L received a constant drip infusion (40 mL/kg/h) consisting of 5 L of isotonic saline spiked with 8.4% $NaHCO_3$.

c Calves with dehydration degree ≥ 2 received an additional 5 L bag of isotonic saline at slow infusion rate (10 mL/kg/h).

Table 5. Logistic regression results for exploring the relationship between medical records and disease outcome in data split according to treatment regimens.

	P value	OR	CI 95%	
Treatment regimen 1				
Serum total protein concentration	0.029	8.78	1.25	61.69
Treatment regimen 2				
Serum total protein concentration	<0.001	3.23	1.77	5.88
Rectal temperature	0.032	1.52	1.04	2.22
Treatment regimen 3				
Serum total protein concentration	<0.001	2.52	1.88	3.34

OR, odds ratio; CI 95%, confidence interval

Treatment regimen. 1 = oral rehydration; 2 = parenteral rehydration with 5 L of isotonic saline spiked with 8.4% $NaHCO_3$ at constant drip infusion (40 mL/kg/h); 3 = parenteral rehydration with additional 5 L of isotonic saline at slow infusion rate (10 mL/kg/h).

Table 6. The predictive values for survival calves of the serum total protein (STP) concentration at 50 g/L, rectal temperature at 38.3 °C, and blood pH at 7.12 cut-off values in 605 diarrheic Holstein Friesian calves.

STP concentration	Value	CI 95%
Sensitivity	71.2%	66.6% to 75.5%
Specificity	71.3%	64.7% to 77.9%
Positive Predictive Value	84.5%	81.2% to 87.3%
Negative Predictive Value	53.2%	49.1% to 57.8%
Accuracy	71.4%	67.6% to 74.9%
Rectal temperature	Value	CI 95%
Sensitivity	61.6%	56.7% to 66.3%
Specificity	55.5%	48.1% to 62.6%
Positive Predictive Value	74.9%	71.4% to 78%
Negative Predictive Value	40.1%	36.0% to 44.4%
Accuracy	59.7%	55.6% to 63.6%
Blood pH	Value	CI 95%
Sensitivity	68.7%	63.6% to 73.5%
Specificity	42.4%	34.8% to 50.2%
Positive Predictive Value	71.4%	68.3% to 74.3%
Negative Predictive Value	39.3%	33.9% to 45.0%
Accuracy	69.2%	55.9% to 64.4%

CI 95%, 95% confidence interval

Table 7. The predictive values of the serum total protein (STP) concentration split according to dehydration score groups in diarrheic Holstein Friesian calves.

	Dehydration score 0 cut-off >45 g/L		Dehydration score 1 cut-off >50 g/L		Dehydration score 2 cut-off >50 g/L		Dehydration score 3 cut-off >55 g/L	
	Value	CI 95%	Value	CI 95%	Value	CI 95%	Value	CI 95%
Sensitivity	83.33%	65.28% to 94.36%	65.93%	57.28% to 73.86%	86.05%	79.95% to 90.85%	82.22%	67.95% to 92.00%
Specificity	78.95%	54.43% to 93.95%	71.11%	55.69% to 83.63%	59.18%	48.79% to 69.01%	60.66%	47.31% to 72.93%
Positive Predictive Value	86.21%	72.06% to 93.81%	87.25%	80.99% to 91.67%	78.72%	74.32% to 82.55%	60.66%	52.32% to 68.41%
Negative Predictive Value	75.00%	56.60% to 87.34%	41.03%	34.02% to 48.42%	70.73%	61.69% to 78.39%	82.22%	70.50% to 89.95%

CI 95%, 95% confidence interval

Figure 1. Receiver operating characteristic curves used to calculate the optimal cut-off points of (A) serum total serum protein concentration (STP; g/L), (B) rectal temperature (°C) and (C) blood pH for predicting outcome in dairy calves affected by neonatal calf diarrhea (NCD). The cut-off points found were >50 g/L for STP concertation, >38.3 °C for rectal temperature and >7.12 for blood pH.

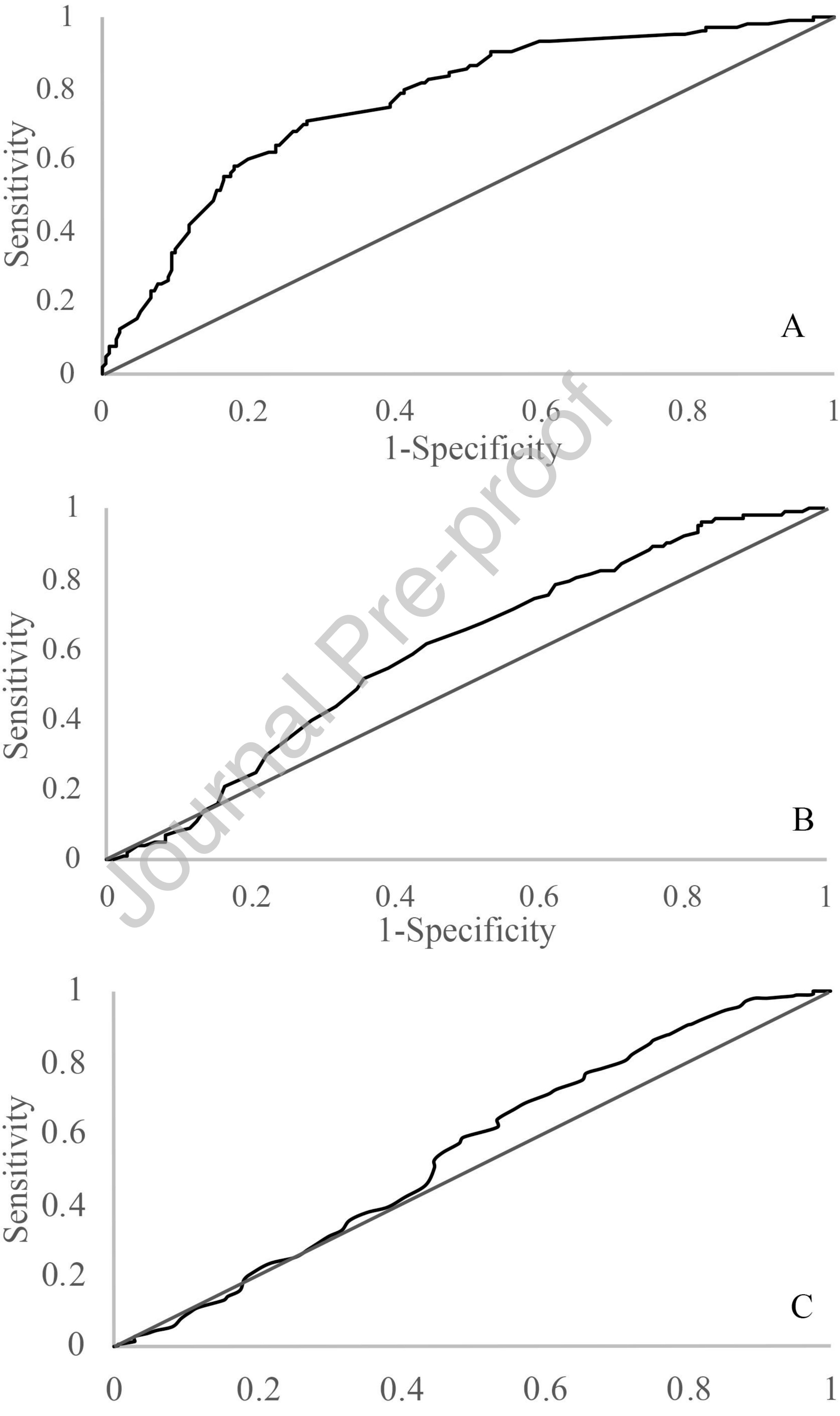
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1-Specificity
A
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1-Specificity
B
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
C

Figure 2. Receiver operating characteristic (ROC) curves used to calculate the optimal cut-off points of total serum protein (STP; g/L) concentration for predicting outcome in dairy calves affected by neonatal calf diarrhea (NCD) in data split according to dehydration scores.

(A) ROC curve of STP concertation in calves with dehydration score 0. Cut-off point 45 g/L.

(B) ROC curve of STP concertation in calves with dehydration score 1. Cut-off point 50 g/L.

(C) ROC curve of STP concertation in calves with dehydration score 2. Cut-off point 50 g/L.

(D) ROC curve of STP concertation in calves with dehydration score 3. Cut-off point 55 g/L.