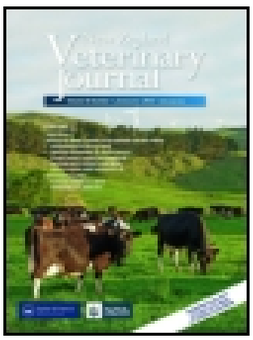

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RESEARCH ARTICLE

Downer cows: a reanalysis of an old data set

KE Lawrence [a], RG Clark[b], HV Henderson [c], K Govindaraju [d] and C Balcomb[a]

[a]School of Veterinary Science, Massey University, Palmerston North, New Zealand; [b]36 Rodeo Drive, Wanaka, New Zealand; [c]Statistics, AgResearch, Hamilton, New Zealand; [d]School of Mathematical and Computational Sciences, Massey University, Palmerston North, New Zealand

ABSTRACT

Aims: To compare the performance of two predictive models for the survival of downer cows.
Methods: The first model had been developed in 1987 using a dataset containing missing values, while the second, new model was developed on the same dataset but using modern data imputation and analytical methods. Missing data were imputed using multiple imputation by chained equations and a logistic regression model fitted to the imputed data, with survival or not as the outcome variable. The predictive ability of the model built on the imputed data was contrasted with the original prognostic model by testing them both on a second smaller but complete data set, collected contemporaneously with the development of the original model but from a different region of New Zealand. Sensitivity, specificity, accuracy, and cut point for the two models were calculated.
Results: The original 1987 model had a slightly higher accuracy than that of the new one with a sensitivity of 0.85 (95% CI = 0.72–0.94) and a specificity of 0.82 (95% CI = 0.7–0.91), using a cut point for the probability of survival = 0.313.
Conclusions: The original prognostic formula published by Clark *et al*. in 1987 performed as well as a modern model built on an imputed data set.
Clinical relevance: The use of a prognostic test based on the Clark model should remain an important part of the clinical examination of downer cows by New Zealand veterinarians.

Abbreviations: AUC: Area under the curve; AST: Aspartate transaminase activity; CK: Creatine phosphokinase activity; GAM: Generalised additive model; NSAID: Non-steroidal-anti-inflammatory drugs; PCV: Packed cell volume

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Introduction

Recumbent cows can be described as either down or downer cows, which is confusing. A down cow is often defined as a cow that has been recumbent for more than 12 hours, whereas a downer cow is said to be an animal which has been recumbent for more than 24 hours but remains bright, alert, and responsive, with no obvious signs of systemic disease (Cox *et al*. 1986; Poulton 2015). It is not altogether clear from a clinical perspective how useful this distinction is, and so in this article recumbent cows will only be referred to as downer cows. Most cases of downer cows occur within 48 hours of calving (Fenwick 1969), but they can also occur pre-calving or at any stage of the lactation, e.g. at mating. However, the downer cow definition used in this article will be restricted to those cows that were recumbent in the periparturient period.

Confusion over the use of the terms down and downer means that interpretation of survey data from within and between countries is often difficult. However, there is widespread agreement that the pathogenesis of downer cows is a mixture of primary causes and secondary complications (Cox 1988; Andrews *et al*. 1992; Poulton *et al*. 2016a). The primary cause is the initial problem which puts the cow on the ground. Secondary complications are those which keep the cow on the ground, beyond when recovery from the primary cause would reasonably have been expected to occur (presuming that recovery from the primary cause is possible, given the correct diagnosis and appropriate treatment).

The primary causes of a periparturient downer cow include metabolic parturient paresis, skeletal injury from dystocia or trauma, neuromuscular injury from dystocia or trauma and toxic peri-parturient disease such as mastitis or metritis. The secondary complications are often neuromuscular with pressure damage – ischaemic necrosis – to muscles and nerves of the hind and fore limbs or secondary to the cow struggling to get up. Secondary complications are highly likely for any recumbent cow, no matter what the primary cause, but the speed and severity of the secondary complication is heavily dependent on the quality of care and nursing (Chamberlain and Cripps 1986; Huxley 2006; Poulton *et al*. 2016b).

CONTACT KE Lawrence k.lawrence@massey.ac.nz
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A consistent pattern found in larger (>200 cows) downer cow studies is that only about a third of downer cows become ambulatory again (i.e. survive). Studies from the USA (Cox *et al*. 1986), New Zealand (Clark *et al*. 1987a), Australia (Poulton *et al*. 2016a) and Canada (Labonte *et al*. 2018) report rates of 33%, 39%, 32% and 37%, respectively. This unfortunately means that two-thirds of downer cows potentially suffer unnecessarily and if identified earlier could be humanely euthanised. Puerto-Parada *et al*. (2021) found 55% survival to discharge from a retrospective study of 1,318 hospitalised downer cows from 1994 to 2016. The better outcomes reported by this study could be due to the use of cow flotation tanks, however, these were mostly referral cases and those cows diagnosed with fatal musculoskeletal conditions were excluded from the analysis. It is also interesting to note that despite the widespread availability of non-steroidal-anti-inflammatory drugs (NSAID) for cattle since the two earliest studies in 1986 and 1987, there has been no noticeable improvement in survival rates for downer cows.

The treatment of downer cows can be frustrating, expensive, and time consuming, for both the dairy farmer and veterinarian involved. A competent dairy practitioner, following a thorough and systematic clinical examination, should be able to correctly identify and euthanise those animals with a hopeless prognosis. These animals will usually include those affected with limb/pelvic fractures, hip dislocations, severe neuropathies, and severe toxic conditions such as gangrenous mastitis or salmonellosis. This, however, still leaves many cows for which the definitive diagnosis and prognosis is difficult to determine, and where the impact of adequate nursing and supportive treatment is unclear.

The welfare implications for downer cows are obvious, and the use of biochemistry tests as prognostic indicators is, in these situations, invaluable (Andrews 1993; Shpigel *et al*. 2003, Puerto-Parada *et al*. 2021). However, downer cows are a clinical manifestation of a complex, multifactorial syndrome, the determinants of which are likely to differ between systems, seasons, countries, and farms. Researchers on downer cows in Greece and Germany have found an increased association with botulism or fatty liver (Kalaitzakis *et al*. 2010; Rulff *et al*. 2015), which demonstrates the range of the possible aetiologies involved in this condition. Given this, the performance of any prognostic test is likely to be specific to the system where the data were collected, because prognosis is determined by the unobserved relationship between a measured factor and a measured outcome that is dependent on the unmeasured particularities of the system. Therefore, it is necessary to quantify the relationship between predictors and outcome under New Zealand conditions.

A prognostic profile for downer cows based on two biochemical tests, aspartate transaminase (AST) activity, and urea concentration in serum, and the duration of recumbency at blood sampling, was developed 35 years ago in New Zealand (Clark *et al*. 1987a) and has been used by New Zealand veterinarians since then to make rational clinical decisions on treatment and prognosis for downer cows. However, at present the use of downer cow profiles by practitioners in New Zealand appears to be limited, with only approximately 200 profiles requested by clients of Gribbles Veterinary and SVS Laboratories each year for the last 5 years (B. Vaatstra[1] and S. Forsyth,[2] pers. comm.).

Remarkably, the original data set for Clark *et al*. (1987a) is still in existence but contains several missing data points. Even more remarkable is the existence of a second data set collected over a similar era (Sutherland 1984). The prognostic model of Clark *et al*. (1987a) was evaluated on the Sutherland data, using statistical methods of the time, and reported in conference proceedings (Clark *et al*. 1987b). However, the evaluation reported by Clark *et al*. (1987b) did not estimate a cut point for the probability of survival, which maximised the ability of the original prognostic model (Clark *et al*. 1987a) to differentiate between cows that survived and those that did not.

The aim of this study was to re-analyse the original data sets, which included varying amounts of missing data, use modern data imputation methods to impute the missing data and develop an alternative model to that developed by Clark *et al*. (1987a) for predicting survival in periparturient downer cows. The predictive ability of the new model would then be contrasted with the original Clark model (Clark *et al*. 1987a) by testing both on the Sutherland data set, and sensitivity, specificity, accuracy, and a cut point for the two models calculated.

Materials and methods

Clark data set

The original data set will be referred to as the Clark data set. This had been collected in 1983 (164 samples) and 1984 (352 samples) and constitutes blood samples taken by veterinarians from downer cows and submitted to Ruakura Animal Health Laboratory (Hamilton, NZ). The Clark data set has data for 516 cows, of which 166/516 (32.2%) survived (stood up), 127/516 (24.6%) died, 142/516 (27.5%) were euthanised and 81/516 (15.7%) for which the outcome was

[1]B. Vaatstra, Gribbles Veterinary, Palmerston North, NZ.
[2]S. Forsyth, SVS Laboratories, Hamilton, NZ.

missing. There are 12 recorded variables in the data set: the laboratory accession number; calving (1 if the cow was down before calving, 2 if down after calving); number of days recumbent when blood sampled; serum creatine phosphokinase activity (CK, U/L at 30°C) (measured in 1983 at a temperature of 25°C and retrospectively converted to an equivalent measure at 30°C); serum AST activity (U/L at 30°C) (measured in 1983 at a temperature of 25°C and retrospectively converted to an equivalent measure at 30°C); serum urea concentration (mmol/L) (measured in 1984 only); urinary ketones (+, ++ or +++); packed cell volume (PCV, %); serum calcium concentration (mmol/L); presence of inflammation (0 = no, 1 = yes); presence of myopathy (0 = no, 1 = yes); and outcome (died, euthanised or survived, where survived means that the cow became ambulatory again).

Inflammation was considered present when the total protein:fibrinogen ratio was < 10:1 and/or white cell changes consistent with neutropaenia and/or left shift were observed. Myopathy was diagnosed if the AST or CK activity exceeded critical values, which were themselves dependent on the number of days recumbent at sampling (Clark *et al*. 1987a). Urea was only added to the biochemistry panel from 1984, which meant this analyte was not measured for those samples submitted in 1983.

Sutherland data set

The second data set will be referred to as the Sutherland data set. It had been collected between 7 May 1984 and 8 October 1984 and constitutes blood samples collected by practitioners from downer cows and submitted to Whangārei Animal Health Laboratory (Whangārei, NZ) (Clark *et al*.1987b). The Sutherland data set has many more measured variables than those recorded in the Clark data set, but importantly, from 1984 onwards it has the same variables as Clark collected. Time recumbent at sampling was recorded in hours and not days, but with the same three outcome categories, died, euthanised or survived. The Sutherland data set has no missing data and comprises110 cows of which 48/110 (43.6%) survived, 31/110 (28.2%) died, and 31/110 (28.2%) were euthanised.

Processing of datasets

For the imputation and analysis, the outcome variable, for both data sets, was converted to a binary categorical variable, where if the cow survived (i.e. stood up) = 1 and if the cow died or was euthanised = 0.

To populate the two variables "inflammation" and "PCV", a blood sample collected into a tube with anticoagulant is required. Since it was preferred that the proposed prognostic profile should be completed using a single serum sample, inflammation and PCV were not included in the analysis for either dataset. Furthermore, the variable "myopathy" was derived from CK and AST, variables that would themselves be tested in the model, so this variable was not included in the analysis either.

The AST and CK activities were measured at 30°C for the Sutherland data (Clark *et al*.1987b) and Clark 1984 data, whereas in the Clark 1983 dataset, they were measured at 25°C (Clark *et al*. 1984) and converted to 30°C for the Clark *et al*. (1987a) publication (RG Clark,[3] pers. comm.). Only the data collected by Clark in 1984 included urea concentration and had been analysed using the same test temperature (30°C) and Hitachi analyser as Whangārei Animal Health Laboratory. Consequently, only this Clark 1984 data (used in Clark *et al*.1987b) were used for data imputation and the alternative model construction.

The final reduced Clark data set used for data imputation had 352 cows and six variables, calving, days recumbent at sampling, AST activity, CK activity, urea concentration and outcome.

The Sutherland dataset contained the same variables but the hours recumbent at sampling for the Sutherland data set were divided by 24 to give days recumbent at sampling.

Processing of existing model

The original Clark *et al*. (1987a) model was built using a correction factor for AST of 1.37 so the intercept value for this model, 3.612, was adjusted to 3.278 using the following formula:

$$3.278 = 3.612 - 1.0625 \times \log(1.37)$$

where −1.0625 is the coefficient for log(AST) from the original model and log is the natural logarithm. This adjustment was necessary to ensure that the AST data used to build the imputed model and the original Clark model were on the same scale.

Statistical analysis

Relationship between CK, AST, urea, and days recumbent

It was suspected that the relationship between the biochemical parameters (CK, AST, urea) and days recumbent at sampling would be non-linear and vary depending on the outcome for the cow. Consequently, scatterplots of CK, AST and urea against days recumbent at sampling, categorised by outcome, were prepared, combining the data from the Clark 1984 and Sutherland studies. Smoothed generalised additive model (GAM) regression lines were then fitted to the

[3]R.G. Clark, 36 Rodeo Drive, Wanaka, New Zealand.

plots, following Wood (2011), to capture the relationship between the measured analyte and days recumbent for each outcome (survived, euthanised, died). Unlike for loess smoothing, which does not require *a priori* specification of the relationship between the response and the predictor variables, GAM smoothing does specify that the response variable partly depends on the predictor variable. This results in less irregular smoothing patterns, that are often seen with loess smoothing, and is becoming the preferred method to assess the relationship between variables at data exploration.

Bivariate relationship between CK, AST, urea, days recumbent and probability of survival

To explore the change in probability of survival with increasing CK, AST, urea, and days recumbent at sampling, bivariate plots of the probability of survival against CK, AST, urea, and days recumbent at sampling were prepared, with fitted smoothed regression lines from a GAM model using a logistic link function. A univariate GAM model was fitted for each of the four predictor variables. A GAM was used rather than a generalised linear model (GLM) to explore the relationship between each predictor variable and the probability of survival, because we again suspected that the relationship with the logit of the probability of survival would be non-linear. It was believed that the results of these plots would aid variable selection for the final predictive model.

Data analyses were performed in R v4.1.1 (R Core Team 2021, R Foundation for Statistical Computing, Vienna, Austria).

Data processing and predictive multivariable model construction

Data missingness

Data missingness was summarised for the six variables in the reduced Clark 1984 data and plotted.

Data imputation

The missing dichotomous categorical variables were imputed using logistic regression and the missing continuous variables were imputed using predictive mean matching. All imputations were completed following Van Buuren and Groothuis-Oudshoorn (2011), using multiple imputation by chained equations (MICE), and 500 complete data sets were imputed for model building. Briefly this method of imputation uses chained equations, in which an imputation model is specified separately for each variable, using the other variables as predictors. At each stage of the algorithm, an imputation is generated for the missing variable, then this imputed value is used in the imputation of the next variable. This process repeats until the algorithm converges (Horton and Kleinman 2007). Prior to imputation the values of CK, AST and urea were normalised by log transformation and subsequently the missing data for these variables were imputed on the log scale. Predictive mean matching is a non-parametric method of data imputation which offers substantial advantages over simple linear regression alone. For each variable, predictive mean matching calculates the predicted regression values for its non-missing and missing observations from other variables in the data set. It then fills in a missing value by randomly selecting one value from a subset of the non-missing observations, typically $n = 3–5$, whose predicted values are closest to the predicted value for the missing observation (Hong and Lynn 2020). This way the missing values are repeatedly replaced with plausible values that are already present in the data set, which removes reliance on a single imputed value and accounts for uncertainty in their estimation (Enders 2010).

Imputed data predictive multivariable model construction

A separate GLM multivariable logistic regression model with the binomial outcome, survived or not, using the predictor variables calving, days recumbent at sampling, AST activity, CK activity, urea concentration, biologically plausible interactions and polynomials and a logit link, was fitted to each of the 500 imputed Clark data sets. Summary pooled coefficients for the 500 separate models were derived by pooling likelihood ratio statistics (Meng and Rubin 1992) and following a methodology developed by Heymans (2020). Variables were retained in the pooled model at $p < 0.05$ using a backwards selection algorithm. To explore other methods of capturing the non-linearity of urea, a second GLM model with a logit link was fitted to the imputed data using a cubic spline for log(urea) with 3 knots, instead of a polynomial term. Goodness of fit for the two pooled models was assessed using the area under the curve (AUC), the Hosmer Lemeshow statistic and Nagelkerke's R-squared. A logistic GLM with polynomial or spline terms was preferred over a logistic GAM, to give a more interpretable output and be like the approach used in the original paper. Although the use of spline terms does make the proposed model more flexible that the original Clark *et al.* (1987a) model.

Multivariable model testing

The predictive model from Clark *et al.* (1987a), with adjusted intercept, gave a probability of survival (P):

$$P = 1/\left(1 + \exp\left(-\left(3.278 + 3.704 \times \log(\text{urea}) - 1.235 \times (\log(\text{urea}))^2 - 1.063 \times \log(\text{AST}) - 0.199 \times \text{days recumbent at sampling}\right)\right)\right)$$

where log is the natural logarithm and exp the exponential function. This original Clark model and the

final logistic model built using the imputed data set were each tested on the Sutherland data set, comparing each model's predictions of survival with the observed survival recorded in Sutherland's data. The predictive performance of each model depends on the probability value used as a cut point indicating survival or euthanasia. Receiver operating characteristic curves were generated using every possible cut point value for each model to find the predicted probability of survival, aka cut point, which maximised that model's ability to correctly predict which cows survived and which did not. The cut point for each model was that which maximised the Youden index (sensitivity + specificity − 1) found using a method developed by Thiele and Hirschfeld (2021). At the optimum cut point for each model, the sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and AUC were calculated using techniques developed by Kuhn (2008) and Stevenson *et al*. (2022).

Results

Relationship between CK, AST, urea, and days recumbent

Plots of CK activity, AST activity and concentration of urea against days recumbent when sampled, for the combined Clark data from 1984 and Sutherland data, are shown in Figure 1 with fitted GAM-smoothed regression lines, categorised by outcome. In all three plots, the cows that survived had the lowest activity or concentration of the measured analyte when sampled whereas cows that died had the highest. For those cows that survived, the activity of CK peaked around 3 days, slightly earlier than for cows that died (Figure 1(a)).

Bivariate relationship between CK, AST, urea, days recumbent and probability of survival

Plots for the probability of survival against CK, AST, urea, and days recumbent at sampling are shown in Figure 2. The probability of survival for the combined Clark 1984 and Sutherland data sets shows an almost linear decline with AST activity and a curvilinear decline with CK activity and days recumbent at sampling. In comparison, cows with high and low concentrations of urea had the lowest probability of survival producing an inverted U-shaped curve, although the small number of data points from cows with low urea concentrations makes this observation less reliable.

Data missingness

The reduced Clark 1984 data set included 352 cows, and six variables: calving, days recumbent at sampling, CK activity, AST activity, urea concentration, and outcome, which gave potentially 2,112 data values. Altogether, there were 243/2,112 (11.5%) missing data values, with 68/352 (19.3%) missing data on calving, 71/352 (20.2%) missing data on days recumbent, 9/352 (2.6%) missing CK activity, 8/352 (2.3%) missing AST activity, 6/352 (1.7%) missing urea concentration, and 81/352 (23%) missing an outcome (Supplementary Figure 1). Despite this there were 260/352 (73.9%) cows with complete data.

Imputed data multivariable model

The logistic model built on the imputed data with a polynomial term for log(urea) showed a significant effect of days recumbent at sampling, log(CK), log (AST), log(urea) and an interaction between days recumbent and log(CK) on the probability of a downer cow surviving (Table 1). There was no evidence to support an effect of calving ($p = 0.08$) or a quadratic term for log(urea) ($p = 0.14$). The predictive model fitted to the imputed data gave a probability of survival (Pi):

$$\begin{aligned}\text{Pi} = 1 / (1 + \exp(-(9.531 - 0.529 \times \log(\text{CK}) \\ - 1.098 \times \log(\text{urea}) - 0.681 \times \log(\text{AST}) - 1.261 \\ \times \text{days recumbent at sampling} + 0.139 \\ \times \text{days recumbent at sampling} \times \log(\text{CK}))))\end{aligned}$$

where log is the natural logarithm and exp the exponential function.

Assessment of model fit was satisfactory, with a Nagelkerke's R squared = 0.34, the Hosmer Lemeshow statistic = 0.26 ($p = 0.98$), indicating there was no evidence for a lack of fit and the AUC = 0.81 (95% CI = 0.75–0.85).

The second model fitted to the imputed data using a cubic spline for log(urea) found no evidence to support the use of cubic spline ($p > 0.05$).

Result of testing models on Sutherland data set

The performance of the GLM model built on the imputed data set and the original Clark model on the Sutherland data set at the respective cut points maximising the Youden index are shown in Table 2. Although the Clark model had a slightly higher specificity, positive predictive value, and accuracy than the imputed model, the CI for these measures overlapped suggesting that the two models had a very similar performance.

Discussion

This study found that a logistic model built using the imputed data did not improve the accuracy of predicting survival in downer cows over the original Clark model, when applied to the independent Sutherland

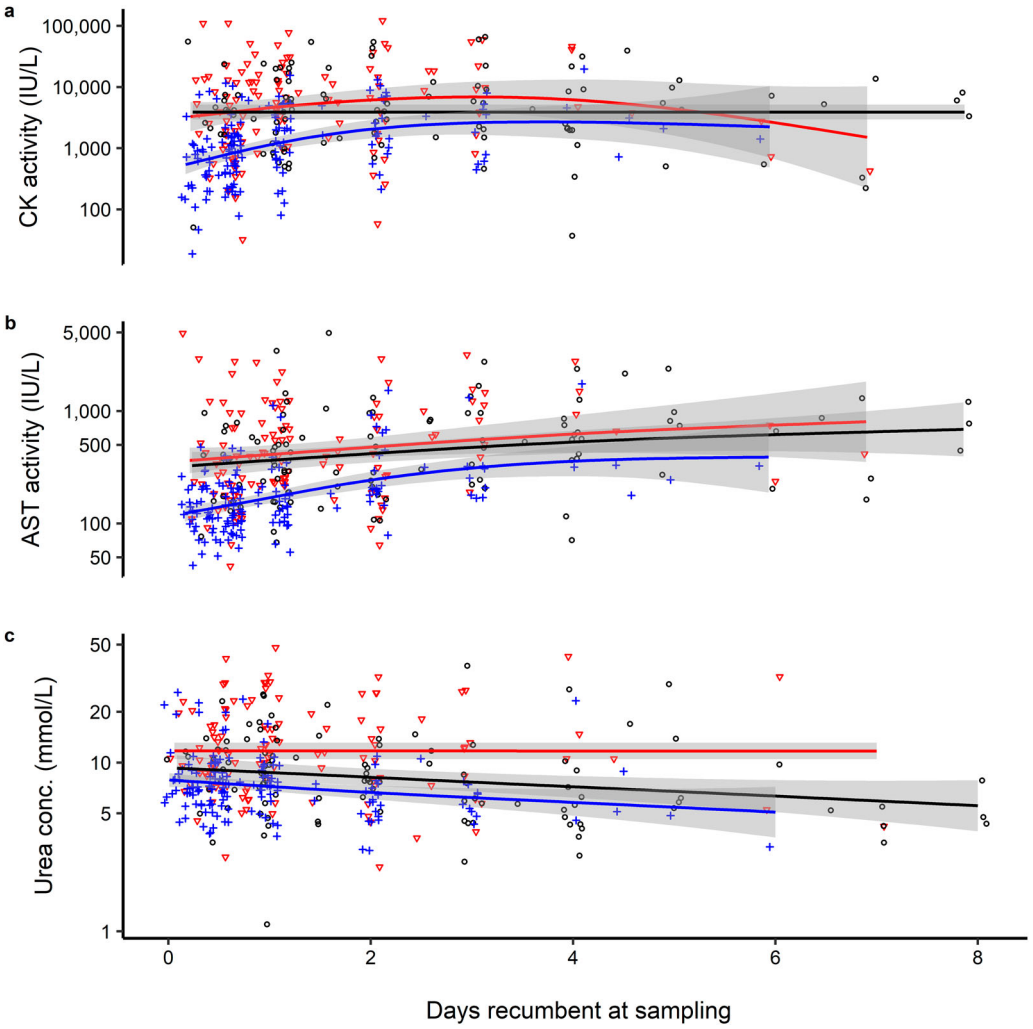

Figure 1. Changes in serum analyte activity or concentration against days recumbent when blood sampled (jittered), for downer cows from combined Clark 1984 data and Sutherland data (Clark *et al.* 1987b). With (a) activity of creatine phosphokinase (IU/L) when sampled, (b) activity of aspartate transaminase (IU/L) when sampled, (c) concentration of urea (mmol/L) when sampled. Generalised additive model smoothed regression lines (blue line and + = survived, black line and ○ = euthanised, red line and ▽ = died) are fitted with 95% CI. Note y axis is on the log scale for all three plots.

data set. This result was probably not unexpected since despite all the inherent missing data, the original Clark model was still based on 254 complete cases (Clark *et al.* 1987a), a comparatively large data set for downer cow studies. Furthermore, as both the original Clark model and the new model based on the imputed data included the variable urea concentration, this meant that both models were restricted to data collected only in 1984, as urea concentration had not been measured in 1983, which also could explain the similar results for both models.

Considering all the possible outcome scenarios for this analysis, a validation of the original Clark model is the preferred result. This justifies the use of the Clark model for predicting the probability of survival for a New Zealand periparturient downer cow for the last 35 years and should encourage practitioners to continue or start using it. The new analysis presented here does add to the original work by finally estimating the threshold predicted probability at which a cow is designated a survivor, i.e. a cut point, for the original Clark model and by providing an estimate of its sensitivity and specificity using an independent data set. The lack of a cut point was given as a reason why Huxley (2006) no longer routinely ran prognostic profiles. These results show that using a cut point for the probability of survival = 0.313 gives a sensitivity of 0.85 and a specificity of 0.82, which is a surprisingly high result and possibly as good as many ELISA tests commonly used as diagnostic tests in New Zealand.

If we think more deeply about the Clark predictive model, it is likely that we are modelling farmer nursing care as a latent variable. Poulton *et al.* (2016b) and Poulton (2020) showed conclusively that poor nursing, negatively impacts survival with one of the most common outcomes of poor nursing being ischaemic muscle necrosis. If we put this into the context of how a practitioner will use the prognostic profile, we suggest the following approach.

The veterinarian who examines a downer cow, first conducts a full medical and comprehensive neuromuscular examination of the cow looking for both

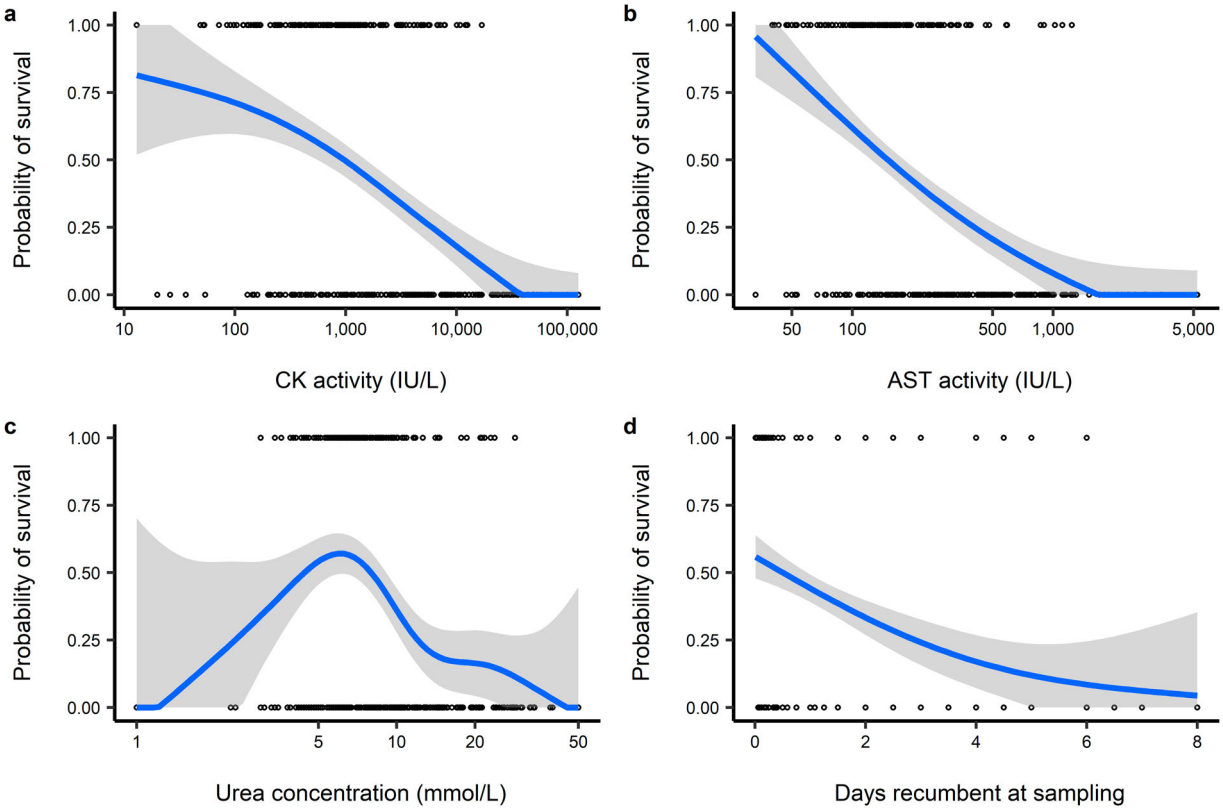

Figure 2. Probability of survival for recumbent cows from combined Clark 1984 data and Sutherland data (Clark *et al.* 1987b), with (a) activity of creatinine phosphokinase (IU/L) when sampled, (b) activity of aspartate transaminase (IU/L) when sampled, (c) concentration of urea (mmol/L) when sampled and (d) number of days recumbent when sampled. The x axis is on the log scale for Figures 2a–c. Generalised additive model smoothed regression lines (blue line) are fitted with 95% CI and the data points (open black dots, at probability = 1 and probability = 0 on the y-axis) are the observed outcomes being the animals which survived or died or were euthanised, respectively.

primary causes and secondary complications. If they are unable to find anything significant, then a single serum sample is taken to establish whether ischaemic muscle necrosis is a possible diagnosis. A downer cow profile is requested from the veterinary laboratory to include AST activity, CK activity and urea concentration and the number of days recumbent at sampling is included in the history. The laboratory compares the CK and AST activities against the time-adjusted critical level for these analytes. If they are above these levels then ischaemic muscle necrosis is confirmed, and the animal likely has a < 5% chance of survival and should be humanely euthanised. If the critical values are not exceeded then the Clark prognostic model is run using the cow's AST activity, urea concentration, and days recumbent to give a probability of survival. An excel spread sheet with the formula is included in the Supplementary Material to this article.

Table 1. Pooled coefficients from individual multivariable logistic regressions fitted to 500 imputed data sets and predicting the logit probability of survival based on data collected by Clark in 1984 (Clark *et al.*1987b) from periparturient dairy cows (n = 352) that had been recumbent for >24 hours.

Variable[a]	Estimate	SE	p-value
Intercept	9.531	1.592	<0.001
Days recumbent at sampling	−1.261	0.493	0.011
Log (CK)	−0.529	0.190	0.006
Log (AST)	−0.681	0.294	0.021
Log (Urea)	−1.098	0.320	0.001
Days recumbent at sampling x log(CK)	0.139	0.066	0.037

[a]AST = aspartate transaminase activity in serum (U/L at 30°C); CK = creatine phosphokinase activity in serum (U/L at 30°C); Urea = serum urea concentration (mmol/L).

The model identifies useful characteristics of the population of cows that do get up: 85% of cows that do get up are predicted within the model to have a survival probability of ≥31.3%. This suggests that the model is useful in differentiating characteristics of those that survive from those that do not. However, this sensitivity is for the population of cows that did get up and, if the nursing is poor then a severe secondary complication could still develop and prevent the cow standing. Recommendations for best practice nursing of recumbent cows are found in Huxley (2006) and Poulton (2020). The accuracy of the prognostic test is reliant on the cow not having a primary cause or secondary complication, such as a fracture or dislocation, that would preclude it from ever standing again, whatever the level of nursing. It is therefore extremely important that any veterinarian using the prognostic profile has carried out a thorough clinical examination and only takes a blood sample to

Table 2. Predictive performance of the multivariable models predicting the logit of the probability of survival for periparturient dairy cows that had been recumbent for > 24 hours. Estimates and 95% CI from the results of testing the generalised linear model built using imputed data and the original Clark model[b] on the Sutherland data.[a]

Model	Cut point	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Imputed	0.21	0.861	0.90 (0.77–0.97)	0.73 (0.60–0.83)	0.72 (0.59–0.83)	0.90 (0.78–0.97)	0.80 (0.71–0.87)
Clark	0.313	0.859	0.85 (0.72–0.94)	0.82 (0.7–0.91)	0.79 (0.65–0.89)	0.88 (0.77–0.95)	0.84 (0.75–0.9)

[a]Clark *et al.* (1987b).
[b]Clark *et al.* (1987a).
AUC = area under the curve, PPV = positive predictive value, NPV = negative predictive value.

determine the cow's prognostic probability once they have found no other clinical symptoms that could limit the cow's chance of survival. If they encounter a cow with a favourable prognosis (probability > 0.313), which does not stand after 3 days, then the practitioner should repeat their examination, since further secondary damage or development of another primary problems such as aspiration pneumonia or toxic mastitis may have occurred since the previous examination, re-evaluate their diagnosis and repeat the prognostic test. For a description of a thorough and systematic clinical examination of a downer cow see Huxley (2006).

One of the potential limitations of the study was that there were three outcome categories for each downer cow, survived, died, or euthanised, which for the purposes of the GLM model were converted to a binary outcome, survived or not. This has led to the concern that some euthanised animals may have recovered had they been given sufficient time. The predictive results for the Sutherland data set using the Clark model would indicate that this was highly unlikely, with almost the same proportion of euthanised animals (6/31; 19.4%) as those that died (5/31; 16.1%) being predicted to survive. Again, we believe this is a good result and validates the veterinarian's or farmer's decision at the time to euthanise the downer cow.

Although Poulton *et al.* (2016b) observed a poor response from downer cows to NSAID, it is likely that this effect was confounded by the quality of nursing and the stage of the disease process at which NSAID were administered. In the presence of poor nursing and or in the face of established ischaemic necrosis, NSAID are unlikely to be effective as sole treatment. The algorithm is thus potentially useful in identifying cows that are unlikely to get up for prompt and humane euthanasia, with NSAID use targeted for those with a higher predicted probability of survival.

Cox *et al.* (1982) investigating the role of pressure in downer cow syndrome found that for animals that survived (stood), the CK activity peaked earlier at 24 hours *vs.* 48 hours for those that didn't. The GAM-smoothed regression lines indicate that for cattle that survived, the CK activity peaked around 3 days and was much lower up to this point than for cows that died or were euthanised (Figure 1(a)). Clark *et al.* (1987a) developed critical levels for CK activity for each day of recumbency up to 7 days and for AST activity averaged over the first 7 days, above which the prognosis was hopeless, i.e. < 5% of downer cows survived. The critical values for CK were 18,600 IU/L on Day 1, 16,300 IU/L on Day 2, 14,000 IU/L on Day 3, 10,900 IU/L on Day 4, 8,500 IU/L on Day 5, 6,200 IU/L on Day 6 and 3,900 IU/L on Day 7 (Table III, Clark *et al.* 1987a). The critical value for AST was 890 IU/L and was applied uniformly for the first 7 days of recumbency. Interestingly the imputed model found a significant interaction between days recumbent at sampling and CK activity, indicating that the prediction of survival from CK activity, depends on which day CK is measured.

Shpigel *et al.* (2003) found similar critical values for CK and AST activities of 16,000 and 702 IU/L respectively, in an analysis of 262 recumbent Israeli cows. This validates the findings from the Clark *et al.* (1987a) study and indicates that the prognostic profile should only be utilised if neither the critical CK or AST activities for that day of recumbency have been exceeded. Shpigel *et al.* (2003) further developed a very simple prognostic model for Day1 based on AST < 171 IU/L to predict survival, however the sensitivity for this test was only 0.58, far lower than the Clark model at 0.85. Puerto-Parada *et al.* (2021) also found reduced probability of survival for cows with elevated AST > 500 IU/L.

The relationship between urea concentration and the probability of survival is complex (Figure 2(c)) and justifies the inclusion of a quadratic term for log (urea) in the final Clark model. Although, a quadratic term was not found significant for the imputed GLM model. The reference range for blood urea in adult cattle is 2.7–12.3 mmol/L which means that the probability of survival changes even within the normal range. This is difficult to explain, however, the interpretation of blood urea in ruminants is complex and cannot be simply ascribed to renal function and hydration status, as it may also reflect changes in rumen microbial metabolism secondary to inappetence, difficulty accessing feed and the quality of feed provided (Getahun *et al.* 2019). This means that urea may be a less reliable analyte for measuring renal function than creatinine (Issi *et al.* 2016). Serum creatinine concentration was used instead of urea as

an indicator of renal failure by Puerto-Parada *et al*. (2021), who found that creatinine concentration > 116 mmol/L was associated with decreased survival. Similarly, Clark et al. (1987a) found only 9% of cows with a serum creatinine concentration > 130 mmol/L survived, although creatinine was only measured in 74 recumbent cows.

Clark *et al*. (1987a) is not the only downer cow study to have developed a prognostic model for downer cows which does not include CK (Shpigel *et al*. 2003; Puerto-Parada *et al*. 2021). The model based on the imputed data did include CK, however, Clark *et al*. (1987a) did not reject the use of CK altogether since they implemented critical thresholds for CK which were used together with the prognostic model to predict survival. And although, Figure 1 clearly shows that for this data CK is much lower for cows that survived compared to cows that die or are euthanised, it is still possible that CK is a poor predictor of survival, as found by Cox (1988) and Burton *et al*. (2009).

A further limitation of this study is that the Clark model is based on the results of biochemistry tests conducted nearly 40 years ago. It is not known whether the same results would be obtained using today's reagents and biochemistry analysers, so further work may be required to check that the measurement of AST activity, CK activity and urea concentration has not changed significantly in this period. In addition, any further work on improving the performance of prognostic profiles for downer cows in New Zealand should possibly include some assessment of heart damage either using cardiac troponin (Labonte *et al*. 2018) or heart rate > 100 beats per minute (Puerto-Parada *et al*. 2021) and the measurement of serum potassium concentration (Beder *et al*. 2020). All these variables have been found useful in overseas studies but have not been evaluated under New Zealand systems and conditions.

Conclusion

A prognostic model built on an imputed data set did not improve the accuracy of prediction over the original formula published by Clark *et al*. (1987a). The relatively high sensitivity and specificity achieved by the Clark prognostic model should stimulate the more widespread application of the formula when veterinarians are managing downer cows in New Zealand.

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ORCID

KE Lawrence http://orcid.org/0000-0002-2453-1485
HV Henderson http://orcid.org/0000-0001-9660-5298
K Govindaraju http://orcid.org/0000-0001-9403-7733

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Lawrence, KE

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