

Preoperative and Postoperative L-Lactatemia Assessment for the Prognosis of Right Abomasal Disorders in Dairy Cattle

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Background: Preoperative L-lactatemia and heart rate have been suggested as prognostic indicators of outcome for cows with right dilatation of the abomasum or volvulus (RDA/AV). However, postoperative L-lactatemia has not been assessed as a potential prognostic tool.

Objectives: To determine the prognostic value of postoperative L-lactatemia (LAC₂), duration of treatment (Dt), relative L-lactatemia difference (compared with preoperative L-lactatemia [LAC₁]) ([LAC₂ – LAC₁]/LAC₂) and change in L-lactate over time ([LAC₂ – LAC₁]/Dt) as compared to preoperative findings (LAC₁ and heart rate [HR]) as prognostic factors in dairy cows with RDA/AV.

Animals: A total of 41 dairy cows were included: 19 with AV and 22 with RDA; 11 cows had a negative outcome (NO) and 30 cows had a positive outcome (PO) based on telephone follow-up with owners 30 days after surgery.

Methods: Prospective cohort study. Analysis was performed using logistic regression and comparison of area under the receiver operating characteristics curve (AUC) using nonparametric tests.

Results: $LAC_1 > 1.4 \text{ mmol/L}$ or $LAC_2 > 2.2 \text{ mmol/L}$ had the same accuracy with sensitivity of 100% (95% CI, 75.1–100%) and specificity of 80% (95% CI, 61.4–92.3%) for predicting NO. The relative L-lactatemia difference ([$LAC_2 - LAC_1$]/LAC_1) or lactate kinetics ([$LAC_2 - LAC_1$]/Dt) were not associated with prognosis. The AUC of the preoperative model (which included HR and lnLAC_1) was 0.92 (95% CI, 0.83–1.0) and that of the postoperative model (including only lnLAC_2) was 0.95 (95% CI, 0.88–1.0); these were not significantly different.

Conclusions and Clinical Importance: Postoperative L-lactatemia is helpful to predict outcome in cows with RDA/AV. The short-term change in blood L-lactate is not a useful prognostic indicator, at least during the period of time spent on the farm for surgery and treatment.

Key words: Biomarker; L-Lactate; Receiver operating characteristic curve.

Surgical abomasal disorders are important in dairy production.¹ Although left displaced abomasum occurs more commonly and has a good prognosis, the prognosis may be more variable in cases of right abomasal disorders, which can be either dilated abomasum (RDA) or abomasal volvulus (AV). The AV generally has a less favorable prognosis than RDA because of various degrees of neurovascular and inflammatory damage that may occur in the abomasum.^{1,2}

For these reasons, objective prognostic tools are required for adequate management of these cases according to their anticipated outcome. Several parameters have been described as preoperative negative prognostic indicators in AV including tachycardia, duration of inappetence, dehydration, increased serum alkaline phosphatase activity, increased serum creatinine concentration, hyperlactatemia, rumen hypomotil-,[3,4] hypochloremia, and hyponatremia.^a ity, Intraoperative and postoperative findings such as high abomasal fluid content, venous thrombosis and blue or black color of the abomasal serosa, poor appetite

Abbreviations:

AUC	area under the ROC curve
AV	abomasal volvulus
HR	heart rate
J	Youden's index
LAC	L-lactatemia
NO	negative outcome
PO	positive outcome
RDA	right dilated abomasum
ROC	receiver operating characteristic
Se	sensitivity
Sp	specificity

within 3 days also have been described as indicators of a poor prognosis.⁵

Of the potentially interesting biomarkers that have been studied previously, preoperative blood L-lactate concentration have been shown to be increased in cows with AV as compared to those with left displaced abomasum.⁶ L-lactatemia also has been negatively correlated with outcome in both hospital^{a,[4]} and on-farm settings.⁷ Serial L-lactatemia (LAC) assessment also has been mentioned as a valuable tool in the assessment of critical care patients. In a study of horses with emergency conditions, after an initial increase, a significant decrease in L-lactatemia was observed in survivors as compared to nonsurvivors.⁸

In dogs with gastric dilatation volvulus, $\geq 50\%$ decrease in L-lactate concentration 12 hours after admission has been described as a good indicator of survival.⁹ The determination of L-lactatemia after surgical replacement of the abomasum has not been studied previously, but potentially could be useful for the

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assessment of L-lactate dynamics after partial (RDA and early AV) or total (AV) blood flow obstruction has been corrected. Reperfusion of the ischemic abomasum may increase L-lactatemia, which also can be of prognostic value as an indicator of abomasal damage.^{6,10} Although not conducted in cattle, these previous studies have stimulated interest in following L-lactate dynamics in cows with right abomasal diseases. From a practical point of view, because this surgery is most often performed in a farm setting where time restrictions occur, it may be valuable to assess the use of postoperative lactate measurement as a prognostic tool in cows with right abomasal disorders.

The primary objective of this study was to assess the accuracy of LAC analysis postoperatively in cows with RDA/AV using single postoperative LAC as well as relative L-lactate difference or L-lactate dynamic compared with preoperative prognostic variables (eg, HR and preoperative LAC). Our hypothesis was that LAC assessment after treatment of RDA/AV or LAC dynamics compared with preoperative LAC would have better accuracy to predict outcome in cows than a preoperative model with HR and preoperative LAC measurements.

Materials and Methods

This project was approved by the Ethics Committee of the Faculté de Médecine Vétérinaire, Université de Montréal. Cows from dairy farms cared for by the bovine ambulatory clinic, Faculté de Médecine Vétérinaire, Université de Montréal, with a surgical diagnosis of RDA/AV from 2010 to 2011 were included in this prospective study. These animals were a subsample of a larger study performed to investigate the preoperative impact of lactatemia on prognosis in cows with RDA/AV.7 The definitions of RDA and AV cases were based on visual and palpation findings during right flank standing laparotomy. An RDA was defined as a simple dilatation of the abomasum by gas, fluid or both without rotation on its mesenteric axis. An AV was defined as dilatation of the abomasum by gas, fluid or both with a counterclockwise rotation when viewed from the rear and a second counterclockwise rotation when viewed from a dorsal position.5 These diagnoses were confirmed by palpation of the rotation axis during surgical exploration and by the manipulation required during the surgical procedure. This subsample was part of a larger ongoing research project based on a study published in dogs during the trial that mentioned interest in postoperative L-lactatemia analysis.9 Before any treatment, a standard physical examination was performed including preoperative HR and procedures to establish clinical suspicion of RDA/AV (eg, abdominal percussion, auscultation, and succussion^{1,2}). A preoperative blood sample was drawn from the caudal vessel and LAC (preoperative L-lactatemia [LAC₁]) was determined immediately using a handheld L-lactate meter,^a which had been validated previously in cattle.^{7,11,12} This meter has a linear relationship with the reference method for L-lactatemia at concentrations <15 mmol/L (which includes the common ranges of L-lactatemia).¹² The time was then noted, the cow treated by a surgical technique using standing right flank laparotomy and eventually with IV fluids (hypertonic saline fluid, or calcium salts) and an antiinflammatory drug according to physical examination findings and the discretion of the veterinary surgeon. After all treatments were administered, a second blood sample was analyzed

for LAC determination (postoperative L-lactatemia [LAC2]); the time between both measurements was noted (Dt). The values of LAC1 and LAC2 were recorded by the producer or the veterinary student so that the clinician was blinded to these results. The outcome of the cows was determined 30 days after surgery by a phone call to the producer by the same investigator (GB).⁴ The same questionnaire was completed during the telephone interview including appetite (0, 25, 50, 75, or 100% compared with herdmates in the same stage of lactation) and milk production (0, 25, 50, 75, or 100% compared with herdmates in the same stage of lactation). A positive outcome (PO) was defined as a satisfied owner with the cow that remained productive in the herd (ie, milk production \geq 75% when compared with herdmates). A negative outcome (NO) was defined as a cow that had died or been culled during the interval or as a producer who was not satisfied with the outcome (ie, performance production below the producer's expectations).⁴

Statistical Analysis

We based our power size calculation on previously published research¹⁰ that described L-lactatemia distribution (mean, 4.8 mmol/L; SD, 2.8 mmol/L) in 15 cows with AV. We wanted be able to detect a difference in mean between NO and PO of at least 3 mmol/L between both groups (with a mean \geq 7.8 mmol/L in NO group). The minimal required number of cows in each group (NO and PO) was 11 with a type II (β) error of 0.2 and a type I (α) error of 0.05 using 1-sided test.

The statistical analyses were performed using commercial software.^{b, c} A chi-squared test was used to compare PO and NO in the RDA and AV groups. Descriptive statistics were calculated for the different variables. The data LAC₁, LAC₂, and Dt, as well as the L-lactate dynamics indices ([LAC₂ – LAC₁]/Dt) and relative L-lactate differences ([LAC₂ – LAC₁]/LAC₁]), were compared for NO and PO cows. Nonparametric Wilcoxon rank-sum analysis was used to compare LAC₁ and LAC₂. Collinearity between LAC₁ and LAC₂ was assessed by calculating the Pearson correlation coefficient (*r*). When multicollinearity was observed, the correlated variables were not used in the same model, but an index including both variables was assessed as recommended.¹³ The level of significance was set at *P* < .05.

Assessment of Diagnostic Accuracy

The accuracy of LAC_1 , LAC_2 ($LAC_2 - LAC_1$)/Dt and ($LAC_2 - LAC_1$)/LAC₁ for predicting NO was determined using a threshold that minimized misclassification with the highest sum of sensitivity (Se) and specificity (Sp) for predicting the NO (Youden's index [J], Equation 1):

$$J = MAX(Se + Sp - 1)$$
(1)

The Se and Sp of thresholds were determined, as was the 95% confidence interval (CI) assuming a binomial distribution.

Logistic Regression Analysis

Two logistic regressions models were built first using a preoperative model (Model 1, with covariates previously reported by Boulay et al⁷) and a complete model (Model 2) that also used postoperative LAC₂. Natural log transformation of LAC₁ and LAC₂ then was performed to improve their normality (because data distribution was skewed to the right).

The 2 logistic regression models were used to model the NO probability (P) in relation to the covariates $(X_1, ..., X_n)$

(Equation 2):

$$\ln(P/(1-P)) = \operatorname{logit} P = \alpha + \beta_1 X_1 + \ldots + \beta_n X_n$$
(2)

Model 1 was the preoperative model using $\ln LAC_1$ and HR as potential covariates as described in previous studies.^{a,[4,7]} Preoperative L-lactatemia and HR were forced into the model because of our previous findings. Model 2 took into account the postoperative values of L-lactatemia using only $\ln LAC_2$ and Dt, indices ($[LAC_2 - LAC_1]/LAC_2$) $[LAC_2 - LAC_1]/Dt$) as potential covariates (with $P \leq .10$ in univariate analysis) that could be used to assess lactate dynamics after medical and surgical treatments. The goodness-of-fit of both models was assessed using the Hosmer-Lemeshow test.¹⁴ The odds ratio associated with both models as well as the 95% CI also were determined.

The area under the receiver operating characteristics curve (AUC) was obtained for each model (concordance (c)-statistic in LOGISTIC procedure in SAS). The prognostic preoperative and postoperative models accuracy then was compared using the non-parametric Mann-Whitney U statistic to compare the 2 ROC AUC.¹⁵ The level of significance was set at P < .05.

Results

Descriptive Statistics

Forty-one cows were included in this study. Of these cows, 19 had AV (10 NO, 9 PO) and 22 had RDA (21 PO, 1 NO). The outcome was significantly different for AV and RDA (P < .01). Descriptive statistics are presented in Table 1. LAC₂ (median, 2.1 mmol/L; range, 0.79-12.9 mmol/L) was significantly higher than LAC₁ range, 0.79–12.8 mmol/L; (median, 1.2 mmol/L;P = .006). Because LAC₁ and LAC₂ were highly correlated (Pearson correlation r, 0.82), they were not used in the same logistic regression model because of multicollinearity. We used the difference between pre- and postoperative L-lactatemia (LAC₂ - LAC₁) according to the total treatment time $([LAC_2 - LAC_1]/Dt)$ or preoperative lactatemia $([LAC_2 - LAC1]/LAC_1)$ as potential covariates.

The J threshold of LAC₁ was established at a cutoff of >1.4 mmol/L. This threshold had a sensitivity of 100% (95% CI, 75.1–100%) and a specificity of 80% (95% CI, 61.4–92.3%) for NO detection. The J

threshold of LAC₂ was established at a cutoff of >2.2 mmol/L and had the same Se-Sp and 95% CI as the LAC₁ cutoff. The accuracy of $(LAC_2 - LAC_1)/Dt$ and relative lactate difference $(LAC_2 - LAC_1)/LAC$ were limited (J thresholds >0.73 mmol/h; Se 54.6% (95% CI, 23.4–83.3%), Sp 90% (95% CI, 73.5–97.9%) and J threshold for $(LAC_2 - LAC_1)/LAC >83\%$ with Se 45.5% (95% CI, 16.7–76.6%), Sp 86.7% (95% CI, 69.3–96.2%), respectively).

Preoperative and Postoperative Models Building

In the different models, only ln-transformed L-lactate results were used. Model 1, with HR and $lnLAC_1$ as covariates, is presented in Table 2. Because of multicollinearity between preoperative and postoperative L-lactate and the fact that no index (relative L-lactate difference or L-lactate dynamics) was associated with outcome, the postoperative model (model 2) only contained $lnLAC_2$ as a covariate.

Comparison of the Preoperative and Postoperative Models

Table 2 summarizes the findings of both prediction models based on the Hosmer-Lemeshow test. Both models fitted the dataset well (P > .05). No significant differences were found between the AUC of the preoperative model (using HR and $lnLAC_1$; AUC₁, 0.92; 95% CI, 0.83–1.0) and the postoperative model (using lnLAC₂; AUC₂, 0.95; 95% CI, 0.88-1.0), indicating that postoperative L-lactatemia was not a better predictor than the preoperative model (Fig 1; P = .41). Both predictive values of the preoperative and postoperative models were plotted in the same figure to allow direct graphical assessment of model performance, as previously recommended (Fig 2).¹⁶ The preoperative model misclassified 3 PO and 2 NO, whereas the postoperative model misclassified 3 PO and 3 NO. The higher AUC of the postoperative model was attributed to the better overall discrimination for the prediction of NO versus PO.

 Table 1. Clinicopathological variables in cows with right abomasal disease with a positive or a negative outcome 30 days after on-farm surgery.

	Median (r	nin–max)	
Variable	Negative Outcome $(n = 11)$	Positive Outcome $(n = 30)$	P-Value
HR	108 (80–160) ^a	80 (60–120) ^a	.005 ^a
LAC ₁	2.8 (1.8–12.9) ^a	$0.85 (0.79 - 5.3)^{a}$.0003 ^a
LAC ₂	6.1 (2.3–12.8) ^a	1.3 (0.79–5.1) ^a	<.0001 ^a
Dt	1.75 (1.0–2.0) ^a	$1.25 (0.75 - 2.5)^{a}$.02 ^a
$(LAC_2 - LAC_1)/Dt$	1.25 (-0.8 to 3.9)	0.15 (-1.3 to 1.9)	.11
$(LAC_2-LAC_1)/LAC_1$	25.0 (-24.2 to 138.9)	15.7 (-4.92 to 163.0)	.43

HR, preoperative heart rate (beats per minute); LAC_1 (mmol/L), preoperative lactatemia determined before any treatment; LAC_2 (mmol/L), lactatemia determined after the abomasal surgery was performed and initial fluid therapy, including hypertonic saline, was administered; Dt (hour), time elapsed between LAC_1 and LAC_2 determination.

^aStatistically different (P < .05).

Variables	OR	95% CI for OR	AUC	95% CI for AUC	P (H-L Fit)	AIC	P-Value
Preoperative r	nodel (model	1)					
HR	1.07	1.00-1.16	0.92 ^a	0.83-1.00	0.44	30.31	.06
lnLAC ₁	13.50	2.03-89.84					.007
Postoperative	model (mode	12)					
$lnLAC_2$	24.98	3.40-183.83	0.95 ^a	0.88 - 1.00	0.81	26.53	.002

Table 2. Evaluation of the two logistic regression models for predicting a negative outcome in 41 dairy cows with a surgical diagnosis of right dilatation/volvulus of the abomasum.

P (H-L Fit): P-value for the Hosmer-Lemeshow fit statistics. P > .05 indicates that there is no indication that the model does not fit the data.

HR, heart rate (in beats per minute); $\ln LAC_1$, natural logarithm of preoperative lactatemia; $\ln LAC_2$, natural logarithm of postoperative lactatemia; OR, odds ratio; CI, confidence interval; AUC, area under the receiver operating characteristic curve for each model; AIC, Akaike information criterion for the complete model (the smaller value indicate a better fit of the model).

^aNo statistical difference was found between both AUC using Mann-Whitney U statistics (Delong et al¹⁵), P = .41.



Fig 1. Receiver operating characteristic (ROC) curves of preoperative and postoperative predictive models in 41 dairy cows with right dilatation of the abomasum or abomasal volvulus. The preoperative model (with heart rate and natural logarithm of preoperative lactatemia) had an area under the ROC curve (95% CI) of 0.92 (0.83–1.00; full line). The postoperative model (including natural logarithm of postoperative lactatemia) had an area under the ROC curve (95% CI) of 0.95 (0.88–1.00; dotted line).

Discussion

L-lactate has been used as a prognostic tool for various diseases and species.^{4,9,17–19} In horses, changes in lactate concentration after intensive care have been recommended as a more reliable prognostic marker than initial measurements.⁸ In dogs, a decrease of \geq 50% in L-lactate concentration within 12 hours after initial treatment is a good indicator of survival.⁹ L-lactate dynamics in this study ([LAC₂ – LAC₁]/D*t*) or relative lactatemia difference ([LAC₂ – LAC₁]/LAC₁) were not associated with outcome, which



Fig 2. Compared predicted probability of negative outcome in 41 cows with right dilatation of the abomasum or abomasal volvulus using preoperative and postoperative models. The predicted probability of each cow with either a positive outcome (circle) or negative outcome (triangle) is plotted for both models. The dotted line indicates the threshold of 0.5 above which a negative outcome is predicted by the model. NO, negative outcome.

precludes a direct assessment of these parameters in our predictive model. However, this finding does not mean that lactate dynamics evolution are not of interest for future studies in cattle with RDA/AV because this study lacks power to detect small differences between groups. On the basis of the differences, we observed in $(LAC_2 - LAC_1)/Dt$ between the 2 groups (mean \pm SD) for cows with PO (0.2 \pm 0.7 mmol/L/h) and NO (1.1 \pm 1.4 mmol/L/h), we would have needed 22 cows with NO and 66 cows with PO to detect this difference as statistically significant (type I error, 5%; type II error, 20%). The relatively small time interval between 2 samplings (maximal 150 minutes) also can explain the lack of significance regarding dynamic L-lactate assessment. The time spent on the farm for treatment primarily includes surgery time. Even in cows with PO, expected L-lactatemia dynamics after abomasal surgery in the first hours represent an increase in L-lactatemia, because lactate concentration in the right gastroepiploic vein is higher than in the

peripheral blood.¹⁰ One of the consequences of abomasal surgery is deinflation of the distended abomasum and restoration of normal blood flow in the case of AV. Reperfusion of the hypoxic abomasum leads to increased L-lactatemia when the blood flow is re-established.¹⁰ L-lactate clearance can be difficult to assess in a field setting during a relatively short initial visit and also because there are no typical revisits 6, 12, or 24 hours after the initial treatment, which are the intervals that have been mentioned previously in other species.^{8,9} Future assessment of L-lactate dynamic should be performed, especially in hospital settings in which serial sampling could be more easily performed than in an on-farm setting. We used coccygeal vessels for establishing L-lactatemia as previously reported.⁴ It was not possible to assess the impact of the vessel sampled (arterial, venous, or mixed) on LAC. However, based on a previous study, peripheral venous samples are a good alternative to arterial sampling.²⁰

The definition of a NO can be seen as a limiting factor for internal or external validation of the study. This definition included both objective (death) and subjective (culling, producer assessment of appetite, and milk production and general satisfaction) parameters. The perception of NO may have been influenced by the farmers' expectations that may differ from 1 farmer to another. Using a systematic description of appetite and milk production during telephone interview was helpful to record a more consistent definition of NO, but objective measurements (eg, milk yield) would have been preferable.

Despite the relatively low number of cases, this study showed that postoperative LAC determination also can be directly used to predict NO in cows with RDA/AV with high Se and Sp when compared with preoperative LAC concentrations. The discriminant value of this test was not significantly different from the prognostic value of a model using HR and preoperative lactatemia from the initial dataset. The small number of cases included in this study may be a cause of this equivocal finding.²¹ One may consider the relative interest of performing only a postoperative measurement of LAC to establish a prognosis for cows with RDA/AV, because at that time, most of the cost of treatment has already been incurred. This consideration may be of interest in 2 clinically relevant situations. First, in highly valuable animals in which LAC_1 indicates a NO, the use of LAC₂ may serve to confirm the preoperative diagnosis with the limitation of highly correlated tests. Secondly, single postoperative LAC determination also would be of interest for a clinician who suspects simple RDA in a cow based on physical examination (the prognosis of which is the same as for a left displaced abomasum^{1,2}), but may ultimately makes a diagnosis of AV during surgery. In this case, the use of a single postoperative assessment of LAC would also be of practical interest to improve the prognostic ability of the clinician. Heart rate also is an inexpensive and rapid diagnostic test that can be easily determined. Unfortunately, it was not possible to record postoperative HR. The prognostic interest of postoperative HR has been described in a previous hospital study.⁵ The authors evaluated postoperative HR after surgery in 80 cows with AV. From day 1 to day 4 postsurgery, tachycardia (HR > 80 bpm) had Se of 36-47% and Sp of 82-92% to predict nonproductive animals (ie, nonsurvival, poor milk production, poor appetite).⁵

The AUC or C-statistic is a nonparametric rank ordering method indicating that cows with a NO taken at random into the dataset had a 91.5% (for model 1) and 94.5% (for model 2) risk of a higher predicted probability of NO than a cow with a PO. With AUC or C-statistic values >0.90, the prediction accuracy of the model is considered to be excellent.²² The interpretation of AUC or C-statistic is not clinically intuitive because it is a global indicator that does not take into account the relative cost of false positive or false negative classifications.¹⁶ Even if the AUC is similar, the 2 models may have significantly different performance because a better determination of 1 stratum of patients at risk could occur in 1 model as compared to the other without any statistically significant difference seen in the AUC. Despite the preoperative model having a lower AUC than the postoperative model, the total number of misclassifications was lower in the preoperative model than in the postoperative model. However, the larger AUC of the postoperative model was attributed to better discrimination of the overall predicted probability depending on the outcome and discrimination between cows with NO or PO. A clinically sound description of the data can also be used, as previously recommended,¹⁶ to directly assess both predictive models by plotting the predicted probability of the 2 models in relation to the observed event. Using this approach and a 50% probability as a threshold for both models (ie, if the logistic regression predicts a probability $\geq 50\%$, this is considered a positive test or vice versa for a negative test). This graphical tool is a simple and intuitive way to compare the initial model to the new model even if both models are nonnested.¹⁶ In the present case, the postoperative model predicted on average very low probability (<0.25) of NO for cows with a PO, and a very high probability of NO (>0.75) for cows with NO. By contrast, the preoperative model predicted more cases (NO and PO) around the decision threshold (0.5)which is the explanation of the lower AUC for the preoperative model.

The dataset used in this study was smaller dataset than that of a previous study published by our group, because it involved modifications to an initial research project and only took into account the preoperative prognostic model.⁷ The cases included in the present study represented situations in which almost 54% (95% CI, 37.4–69.3%) of cows had RDA and 46% (95% CI, 30.6–62.6%) had AV, and 27% (95% CI, 14.2–42.9%) (ie, 5% of NO for cows with RDA and 53% of NO for cows with AV) had an NO probability, which is quite compatible with the larger prospective cohort that we previously described.⁷ Similar findings also have been reported in a previous study (15% NO

30 days posttreatment for cows with RDA and 65% NO for cows with AV).⁴ For these reasons, we believe that our dataset represented an unbiased sample of the larger study on dairy cows with RDA/AV.⁷

In conclusion, this study shows that postoperative L-lactate measurements can be used to predict NO in dairy cows with RDA/AV. The variation in L-lactatemia before and immediately after treatment of the cows was not of prognostic interest in this study, but this finding may have been because of the small dataset and the relatively short interval between the 2 sampling periods.

Footnotes

^a Constable PD, Streeter RK, Koenig GR, et al. Plasma L-lactate and pyruvate concentrations and lactate-pyruvate ratio in 41 cattle with abomasal volvulus. Association of Australian Cattle Veterinarians, Sydney 1998:121–123

^b SAS 9.2, Cary, NC

^c MedCalc software bvba, version 12.7.5.0, Mariakerke, Belgium

Acknowledgment

Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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