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Evaluation of the Glutaraldehyde Test as a Prognostic Indicator for Short- and Long-Term Mortality in Hospitalized Adult Cattle With and Without Sepsis

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ABSTRACT

Background: The glutaraldehyde test (GT) is used as a point-of-care test to detect inflammatory disease in cattle.

Objectives: Describe the relationship between GT and fibrinogen as well as GT and total protein (TP) and determine the ability of the GT to predict death in cattle with and without sepsis.

Animals: A total of 367 hospitalized, adult cattle.

Methods: Clinical and laboratory findings, diagnoses, treatments, and outcome were recorded. Spearman correlation was used to assess the relationship of the GT with fibrinogen and GT with TP in septic and non-septic cattle. Likelihood ratios (LHRs) were calculated, and a receiver operating characteristic (ROC) curve was constructed to identify the most appropriate threshold predictive of death. Logistic regression models were used to assess the predictive ability of the GT at this threshold.

Results: The negative correlation between GT and fibrinogen was stronger in non-septic cattle (rho = −0.7; $p < 0.001$) compared with septic cattle (rho = −0.59; $p < 0.001$). Similarly, GT and TP showed stronger correlation in non-septic (rho = −0.52) than in septic cattle (rho = −0.34; $p < 0.001$, respectively). Non-septic cattle with a GT of ≤ 3 min were 2.7 times (95% confidence interval [CI], 1.79–4.02) more likely to die during hospitalization than cattle with a GT > 3 min. No significant associations were identified between GT results and death in septic cattle.

Conclusions: The GT can be recommended as a valuable tool for predicting death in non-septic cattle but should not be used in acutely septic cattle.

1 | Introduction

Cattle often are presented with nonspecific clinical signs such as decreased feed intake, decreased milk yield, or fever, which can be indicative of many diseases such as mastitis, pneumonia, and traumatic reticuloperitonitis (TRP). Predicting the severity of inflammation and chances of survival using point-of-care tests can be very helpful, especially for clinicians in the field with

Abbreviations: GT, glutaraldehyde test; LDA, left displaced abomasum; LHR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RDA, right displaced abomasum; TP, total protein; TRP, traumatic reticuloperitonitis.

limited access to advanced diagnostics and facing economic considerations that impact therapeutic decision-making in food animal medicine [1].

The glutaraldehyde test (GT, Glutaltest, Dr. E. Graeub AG, Switzerland) is a rapid and inexpensive screening test used for detecting inflammation in adult cattle in several European countries, including Switzerland. It measures the clotting time of glutaraldehyde linking free NH_2 groups of globulins and fibrinogen. Although glutaraldehyde nonspecifically binds any terminal NH_2 group, the GT clotting time has been described as inversely proportional to blood globulin and fibrinogen concentrations [2–4]. A strongly positive GT (< 3 min) has been associated with poor prognosis [5, 6]. Sepsis and the subsequent systemic inflammatory response also increase the mortality of large animals, including cattle [7–9].

In cattle with chronic inflammatory diseases, highly increased gamma globulin concentrations are reported frequently, whereas cattle with acute inflammatory processes often show low gamma globulin concentrations [10]. Hypogammaglobulinemia caused by increased vascular permeability and increased catabolism during sepsis also can occur in patients in septic shock [11, 12]. Similarly, although fibrinogen typically increases during inflammatory diseases, it can decrease because of rapid degradation in acutely septic patients [13, 14]. This observation suggests that the GT may not be a valuable diagnostic tool in cattle with acute inflammatory conditions or sepsis [15–18].

Previous studies focused on investigating the interaction of the GT and different blood variables and its association with specific diagnoses such as TRP [6, 19, 20]. No studies have investigated the difference of GT results in predicting death in cattle with and without sepsis for short-term (hospitalization period) and long-term (follow-up period of 1 year after discharge from the hospital) outcomes. Furthermore, most studies were carried out decades ago and laboratory techniques have evolved substantially since that time [2]. Therefore, reassessment of the GT and its association with blood variables and clinical outcomes is warranted.

Our specific objectives were to: (a) examine the correlation between the GT and fibrinogen and the GT and TP using modern laboratory tests in cattle with and without sepsis, (b) determine the diagnostic test characteristics and prognostic value of the GT regarding the short- and long-term death of hospitalized cattle with and without sepsis and explore a suitable cut-off predictive of death. We hypothesized that the GT would be highly correlated with fibrinogen and TP in cattle without sepsis. Therefore, the GT will be a valuable tool to predict death in cattle without sepsis but not in cattle with sepsis.

2 | Materials and Methods

2.1 | Study Population

Electronic medical records from all cattle presented to the Ruminant Internal Medicine Clinic of the University of Zurich, Switzerland during the year 2020 were reviewed.

Cattle were included if: (a) they were > 1 year of age and (b) the GT, a blood gas analysis, a CBC, and serum biochemistry profile were performed upon admission to the hospital and (c) they were referred for a specific internal medicine complaint. Cattle with primary orthopedic or reproductive complaints were not included in the study because they were admitted by a different service that does not typically perform the GT.

Signalment information was collected from each record: age (years), breed, sex (male, female), pregnancy duration or time since last calving (days), duration of illness before hospital admission, treatment with antibiotics and non-steroidal antiinflammatory drugs (NSAIDs) before admission (yes/no, if yes for how long antibiotics or NSAIDs were given before admission to the hospital).

Recorded physical examination findings included: abnormal temperature at home, at admission to the hospital, and during the hospitalization period (only hyperthermia > 39.0°C or hypothermia < 38.0°C was noted), diagnosis made by the attending clinician, use of antibiotics during hospitalization, type of surgery performed if needed, hospitalization time (days), outcome (survival or death), cause of death (euthanasia, natural death, slaughter) and necropsy results (if death occurred at the hospital).

Data extracted from the CBC results included: leukocytes (10^3 cells/μL), basophils (10^3 cells/μL), eosinophils (10^3 cells/μL), lymphocytes (10^3 cells/μL), monocytes (10^3 cells/μL), absolute neutrophil count (10^3 cells/μL), band neutrophil count (10^3 cells/μL), segmented neutrophil count (10^3 cells/μL), thrombocytes (10^3 cells/μL), PCV (%), erythrocytes (10^6 cells/μL), hemoglobin (g/dL), MCV (fL), MCH (pg) and MCHC (g/dL). Analysis of CBC was performed using a Sysmex XN 1000 (Version 3.07–00, Sysmex Suisse AG, Horgen, Switzerland).

Data extracted from the serum biochemistry profile included: serum total protein (g/L), aspartate aminotransferase (AST; U/L), sorbitol dehydrogenase (SDH; U/L), glutamate dehydrogenase (GLDH; U/L), gamma-glutamyltransferase (GGT; U/L), bilirubin (μmol/L), urea (μmol/L), creatinine (μmol/L), calcium (mmol/L), chloride (mmol/L), magnesium (mmol/L), phosphorus (mmol/L), potassium (mmol/L), sodium (mmol/L), creatine kinase (U/L) and fibrinogen (g/L). The biochemistry profile was performed using a Cobas c501 (Roche Diagnostic AG, Rotkreuz, Switzerland), except for fibrinogen, which was measured using the Start Max (Firma Stago, Glattbrugg, Switzerland).

Blood pH and L-lactate (mmol/L) were recorded from the blood gas analysis, which was performed using the Rapid Point 500 analyzer (Software V2.3.3A, Siemens Healthcare GmbH, Erlangen, Germany).

The GT was performed by mixing equal volumes of glutaraldehyde solution (1 mL of solution contains 12 mg glutaraldehyde and 1 mg disodium EDTA) and whole blood, which was collected from the jugular vein or tail vein [2]. The tube was inverted every 30 s for 10 min. When clot formation was visible, the time was documented in the medical record. If no clot formation was noticed after 10 min, the result was recorded as ≥ 10 min.

2.2 | Outcome Assessment

Short-term death was defined as death during hospitalization. Long-term death was defined as death from the day of discharge until 1 year after discharge. The life status of included cattle was followed in AGATE (Federal Office of Agriculture, Bern, Switzerland), an online registration platform for cattle in Switzerland. Whenever a death was identified for a specific case, the owner was called and asked if the patient had died, been euthanized, or slaughtered. Every patient that died, was slaughtered, or euthanized during hospitalization underwent necropsy and results were recorded.

2.3 | Septic and Non-Septic Cattle

Cattle were identified as septic if they showed toxic changes in neutrophils (toxic granules, Döhle bodies, cytoplasmatic vacuolation) on their blood smear [21–26]. If there was no evidence of toxic changes in neutrophils on the blood smear, cattle were identified as non-septic. Although blood cultures would be needed for a definitive diagnosis of sepsis and identification of the causative pathogen, previous studies demonstrated that examination of blood smears led to the diagnosis of septicemia before the results from bacterial cultures were available and that the number of morphologic changes in neutrophils correlated with the severity of the disease [27].

2.4 | Statistical Analysis

Statistical analysis was performed using R statistical software (Version 4.2.3) and IBM SPSS Statistics (Version 29.0.0.0). Normality of the data was evaluated using the Shapiro–Wilk test and visual examination of histograms. The mean and SD were calculated for normally distributed variables and the median and ranges for non-normally distributed variables. Spearman correlation coefficients (rho) were calculated to assess the relationship between the GT and fibrinogen as well as GT and TP for septic and non-septic cattle. Scatter plots were constructed for data visualization.

Continuous variables were compared between groups (survivors vs. non-survivors) using the Student's *t*-test or the Mann–Whitney *U* test, depending on the normality of the data, whereas categorical variables were compared using the Chi-squared test or Fisher test if the sample size was < 5.

Interval likelihood ratios (LHRs) were calculated for different categories of the GT (0–3, 3.5–6, 6.5–9.5, ≥ 10 min) to identify appropriate thresholds predictive of short- and long-term death. The GT categories were chosen based on previous literature [19]. The interval LHRs for cattle with and without sepsis based on blood test results were calculated separately to determine if there was a difference in predicting the probability of short- and long-term death. The LHR approach initially was used because it provides more information from the dataset that would get lost when dichotomizing continuous data; hence, interval LHR can minimize the risk of distortion when interpreting results in the clinical environment [28–30]. When an interval range contained 0 samples, 1 unit was added to each number for the calculation. The LHR results were considered significant whenever the 95% confidence interval (CI) did not include 1.

Additionally, a receiver operating characteristic (ROC) curve was constructed to calculate the most appropriate cut-off (in minutes) of the GT for determining the risk of death based on the highest sum of sensitivity and specificity, also known as the Youden index (J) [31]. This analysis was performed to provide results that can be better compared to previous studies that used the same methodology.

Test characteristics (sensitivity, specificity, positive and negative predictive values) then were calculated for the identified thresholds for septic and non-septic cattle depending on short- and long-term death to determine whether the GT is useful as a prognostic test. Sensitivity was defined as the proportion of cows that died and were correctly identified by the GT (in our case, a shortened GT ≤ 3 min). Specificity was defined as the proportion of cows that did not die and were correctly identified by the GT (in our case, a long GT ≥ 10 min) [29]. The positive predictive value (PPV) was defined as the proportion of cows classified at a high risk of death by the GT and that actually died. The negative predictive value (NPV) was defined as the proportion of cows classified at a low risk of death that actually did not die. Disease prevalence must be considered when evaluating diagnostic test PPV and NPV and refers to the proportion of cows with a positive GT (in our case, a shortened GT) in relation to the total number of cows in the study population.

A binary logistic regression was performed to determine the effect of different independent and biologically important variables (abnormal temperature, antimicrobial treatment at home and in the hospital, band neutrophils, sepsis, GT, lactate) on predicting the probability of short- and long-term death. All variables with $p < 0.1$ in univariate analysis were included in the model. The variables were entered into the model by forward stepwise selection, and the model that showed the best improvement towards the base model was chosen as the final model. This choice was assessed using Nagelkerke R [2] and the Hosmer-Lemeshow-Test [32, 33]. To evaluate the relevance of sepsis on GT results, decreased logistic regression models also were built with non-septic and septic cattle.

3 | Results

A total of 523 cattle were admitted to the Ruminant Internal Medicine Service of the University of Zurich in 2020. Of these, 367 cattle fulfilled the enrollment criteria for the study and their data were collected. A total of 365 patients were cows and 2 were steers. Included breeds are listed in Table 1. The median age at admission was 4.75 years (range, 1.1–20.8 years). Of all included cows, 125 (34%) were pregnant during the hospitalization period with an average pregnancy duration of 64 days (range, 7–280) at admission.

A total of 124 (34%) cattle had received antibiotics at home and 157 (43%) had received NSAIDs at home before hospital admission. The average duration of illness before referral was 5.7 days (range, 1–350 days; Table S1).

TABLE 1 | Descriptive statistics of the study population ($n = 367$ cattle). The p values for short- and long-term survivors and non-survivors were calculated separately to evaluate any significant differences between the survivor and non-survivor group on admission to the hospital.

	Short-term				Long-term			
Variable	**Number of cattle**	**Survivors ($n = 241$)**	**Non-survivors ($n = 126$)**	***p***	**Number of cattle**	**Survivors ($n = 170$)**	**Non-survivors ($n = 71$)**	***p***
Age (years, range)	367	4.83 (1.41–20.83)	4.75 (1.08–15)	0.66	241	4.83 (1.41–14.83)	5.34 (2.0–20.08)	0.59
Breed				0.001				0.34
Braunvieh	139	78 (32%)	61 (48%)		78	59 (35%)	19 (27%)	
Holstein Friesian	106	76 (32%)	30 (24%)		76	50 (29%)	26 (37%)	
Red Holstein	57	48 (20%)	9 (7%)		48	31 (18%)	17 (24%)	
Others	65	39 (16%)	26 (21%)		39	30 (18%)	9 (12%)	
Sex				0.12				NA
Male	2	0	2 (2%)		0	0	0	
Female	365	241 (100%)	124 (98%)		241	170 (100%)	71 (100%)	
Pregnant				0.70				0.53
Yes	125	87 (36%)	38 (30%)		87	64 (38%)	23 (32%)	
No	215	144 (60%)	71 (56%)		144	99 (58%)	45 (63%)	
Duration of pregnancy (days, range)	122	105 (7–280)	120 (7–280)	0.40	86	105 (7–280)	55.5 (21–270)	0.30
Duration time after calving (days, range)	196	14 (1–150)	14 (1–210)	0.78	133	14 (1–150)	32 (1–210)	0.93

Note: Other breeds include Original Brown Swiss ($n = 19$), Swiss Fleckvieh ($n = 17$), Mixed breeds ($n = 11$), Jersey ($n = 4$), Simmental ($n = 4$), Limousin ($n = 3$), Angus ($n = 2$), Dahomey ($n = 1$), Grauvieh ($n = 1$), Hereford ($n = 1$), Hinterwälder ($n = 1$), Pinzgauer ($n = 1$).
Abbreviation: NA, not available.

The median duration of hospitalization was 7 days (range, 0–34 days; Table S1).

The most frequent diagnoses were left displaced abomasum (17%), pneumonia (15%), TRP (14%), metritis (14%) and small intestinal ileus (14%; Table S2). Most cattle had >1 diagnosis. Of all cattle with GT results ≤3 min ($n = 75$), 70 (93%) had diagnoses that were associated with a chronic inflammatory process (TRP, peritonitis without a visible foreign body, endocarditis, pneumonia, liver abscesses and abscesses from other origins such as retropharyngeal abscess and vertebral abscess). Of the 367 included cattle, 242 (66%) had GT results ≥10 min, and only 75 cattle (29%) had GT results ≤3 min. Of the 75 cattle with GT results ≤3 min, 64 (85%) received antibiotics during hospitalization, and the other 11 cattle were euthanized because of the severity of their disease and poor prognosis. Abnormal temperature at home or in hospital (> 39.0°C or < 38.0°C) was seen in 123 (34%) of all cattle (Table S3).

Of the 367 included cattle, 291 (79%) were treated with antibiotics at home or in the hospital (Table S1). A total of 74 cattle (20.2%) were diagnosed as septic based on the presence of toxic changes in their neutrophils (Table S4).

4 | Correlation of GT With Fibrinogen and GT With Total Protein

There was a moderate negative correlation between GT and fibrinogen (rho = −0.66, $p < 0.001$; Figure 1A1) overall. Sepsis did influence this relationship. When excluding cattle with sepsis, the correlation was strong (rho = −0.7, $p < 0.001$; Figure 1A2), and including only cattle with sepsis resulted in a weaker negative correlation between GT and fibrinogen (rho = −0.52, $p < 0.001$; Figure 1A3) [34].

Moderate negative correlation was found between GT and TP (rho = −0.57, $p < 0.001$; Figure 1B1) overall. Sepsis also influenced this relationship. When excluding cattle with sepsis, the correlation was similar (rho = −0.59, $p < 0.001$; Figure 1B2), but the correlation between GT and TP in cattle with sepsis was poor (rho = −0.34, $p < 0.001$; Figure 1B3) [34].

5 | Short-Term Death

Of the 367 cattle, 241 (66%) survived until discharge and 126 (34%) died during hospitalization. The hospitalization time was

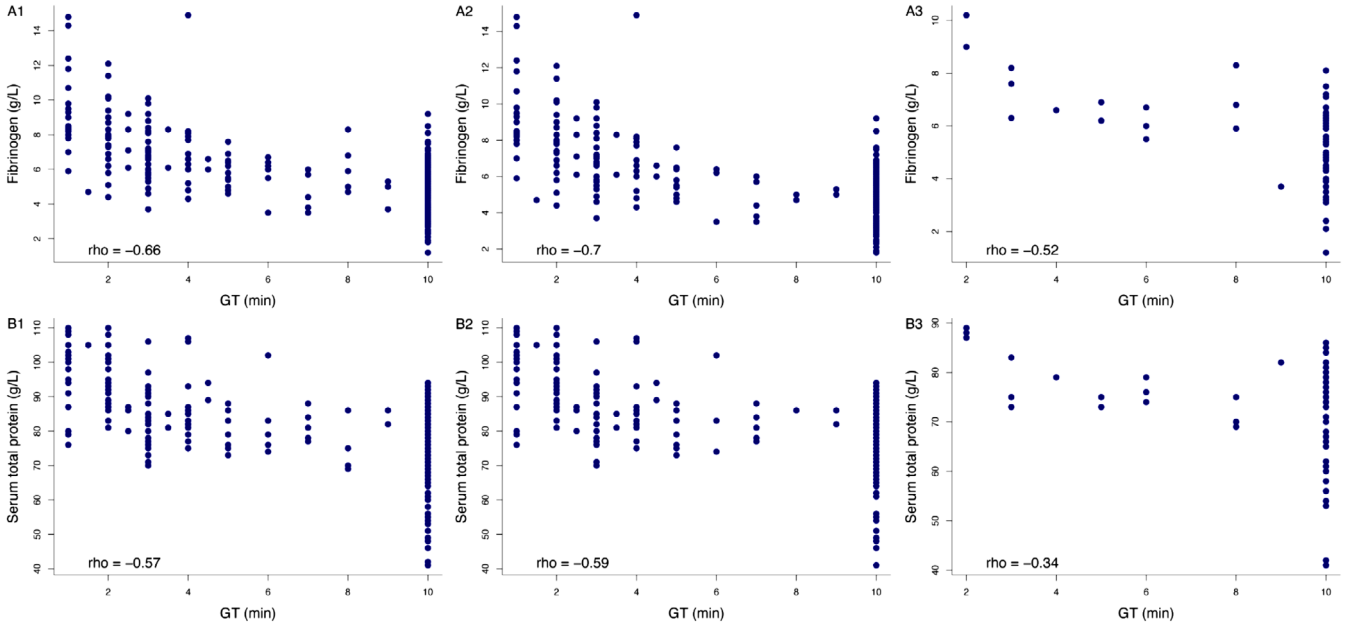

FIGURE 1 | Scatter plots showing the correlation between glutaraldehyde test (GT) results and fibrinogen (A1–A3) and serum total protein (B1–B3) concentrations in 367 hospitalized cows. A1/B1, all cows ($n = 367$); A2/B2, cows without sepsis ($n = 293$); A3/B3, cows with sepsis ($n = 74$); rho, Spearman correlation coefficient.

longer in surviving (median, 8 days; range, 0–32 days) than in non-surviving (median, 2 days; range, 0–34 days; $p < 0.001$; Table S1) cattle. Other than peritonitis, pneumonia, and endocarditis ($p < 0.05$; Table S2), none of the diagnoses were significantly associated with non-survival. Significant differences were found in several of the examined clinical and laboratory variables between survivors and non-survivors (Tables S1–S4). For example, the total number of leukocytes, neutrophils, band neutrophils, fibrinogen, and urea were significantly lower for surviving cattle ($p < 0.05$; Table S4). In contrast, the presence of toxic changes in neutrophils, indicative of sepsis, was significantly higher for surviving than non-surviving cattle ($p = 0.02$; Table S4).

6 | Association Between GT and Short-Term Death

To identify a GT cut off that indicates a higher risk of short-term death, interval LHRs were calculated (Table 2). Overall, cattle with a GT of ≤3 min were 2.2 times more likely to die during hospitalization than cattle with a GT >3 min (Table 2). On the other hand, cattle with a GT ≥10 min were significantly less likely to die than cattle with GT <10 min (Table 2). The results of the LHR analysis were substantially different when evaluating only non-septic cattle versus when evaluating only septic cattle. Non-septic cattle with a GT of ≤3 min were 2.7 times more likely to die than cattle with a GT >3 min (Table 2). In contrast, for septic cattle, no significant results could be found for the different thresholds indicating that the GT is not useful in septic cattle (Table 2).

Diagnostic test characteristics for short-term death for selected thresholds of the GT are presented in Table 3. Based on our ROC curve analysis for a GT threshold of ≤3 min in non-septic cattle, the sensitivity was 0.41, specificity was 0.85, and the Youden Index was 0.26 (Figure 2).

The overall PPV to predict short-term death in our study population with a GT ≤3 min was 35.6% (Table 3). For cattle without sepsis, the PPV was 45.9% and for cattle with sepsis 7.6%. The overall NPV for GT ≥10 min was 70.4%, 70.6% for non-septic cattle, and 78.8% for septic cattle.

Of the 367 animals, 9 were excluded from the final model because of missing values. Thus, only 358 animals were included in the final model.

The following variables were offered to the multivariable regression model based on univariable screening and biological relevance: fever in hospital, use of an antimicrobial, band neutrophils, sepsis, lactate, and GT. Sepsis was not included in the final model because of a non-significant p value ($p = 0.96$).

Accounting for other covariates, cattle with GT results ≤3 min had 4.5 times the odds of short-term death than cattle with GT results >3 min (95% CI, 2.52–8.20; Table 4). Cattle were more likely to die in the short term if they had increased band neutrophils (OR, 2.36; 95% CI, 1.59–3.50) and were less likely to die if they received antibiotics at home or in the clinic (OR, 0.15; 95% CI, 0.08–0.26).

Lastly, to assess the association of GT results ≤3 min with short-term death in the absence of other laboratory data (e.g., in mobile practice or in cases with economic constraints) decreased regression models were built for both non-septic and septic cows. Here, GT results ≤3 min were significantly associated with short-term death in cattle without sepsis (OR, 3.86; 95% CI, 2.19–6.79) but not in cattle where sepsis was present ($p = 0.52$; Table 5).

TABLE 2 | Likelihood ratios for different intervals of the glutaraldehyde test (GT) results associated with short- and long-term death for 367 cows, separated into septic and non-septic.

Short-term death

	All cows ($n = 367$)			Cows without sepsis ($n = 293$)			Cows with sepsis ($n = 74$)		
GT (min)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)
0–3	40	35	2.19 (1.47–3.26)	38	31	2.68 (1.79–4.02)	2	4	0.70 (0.18–2.72)
3.5–6	7	30	0.45 (0.20–0.99)	5	26	0.42 (0.17–1.06)	2	4	0.70 (0.18–2.72)
6.5–9.5	7	6	2.23 (0.77–6.50)	3	6	1.09 (0.28–4.27)	4	0	5.79 (0.71–47.41)
≥10	72	170	0.81 (0.68–0.96)	46	138	0.72 (0.58–0.91)	26	32	0.95 (0.73–1.24)
Total	126	241		92	201		34	40	

Long-term death

	All cows ($n = 241$)			Cows without sepsis ($n = 201$)			Cows with sepsis ($n = 40$)		
GT (min)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)	Non-survivors (n)	Survivors (n)	Likelihood ratio (95% CI)
0–3	8	27	0.71 (0.34–1.49)	7	24	0.74 (0.34–1.61)	1	3	0.83 (0.17–4.10)
3.5–6	10	20	1.20 (0.60–2.43)	9	17	1.24 (0.63–2.82)	1	3	0.83 (0.17–4.10)
6.5–9.5	4	2	4.79 (0.9–25.56)	4	2	5.05 (0.95–26.83)	0	0	1.67 (0.11–25.04)
≥10	49	121	0.97 (0.81–1.16)	37	101	0.93 (0.74–1.52)	12	20	1.03 (0.71–1.49)
Total	71	170		57	144		14	26	

Note: Septic cows are defined as cows that showed toxic changes in their neutrophils on their blood smear. Cows without toxic changes in their neutrophils on their blood smear were defined as non-septic. When an interval range contained 0 samples, 1 unit was added to each number for the calculation.
Abbreviation: GT, Glutaraldehyde test (results in minutes).

TABLE 3 | Test characteristics of different glutaraldehyde test (GT) thresholds predictive of short-term death for cows with and without sepsis.

Short-term death Test characteristics (%)						
	Threshold GT (min)	**Se (95% CI)**	**Sp (95% CI)**	***J***	**PPV[a]**	**NPV[a]**
All cows ($n = 367$)	≤ 3	0.32 (0.24–0.40)	0.85 (0.81–0.89)	0.17	35.6	83.4
	≥ 10	0.43 (0.35–0.52)	0.71 (0.65–0.76)	0.14	42.2	70.4
Cows without sepsis ($n = 293$)	≤ 3	0.41 (0.33–0.52)	0.85 (0.79–0.89)	0.26	45.9	82.0
	≥ 10	0.50 (0.39–0.60)	0.69 (0.62–0.75)	0.19	48.6	70.6
Cows with sepsis ($n = 74$)	≤ 3	0.09 (0.03–0.22)	0.90 (0.76–0.96)	0.00	7.6	90.8
	≥ 10	0.24 (0.12–0.40)	0.80 (0.65–0.90)	0.04	24.9	78.8

Note: In some cases, the Youden Index is 0 because of very low sensitivity and specificity values that would have led to a negative Youden Index.
Abbreviations: GT, glutaraldehyde test; J, Youden Index; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.
[a]PPV and NPV were calculated based on a prevalence of cattle with GT ≤ 3 min of 20% and a prevalence of cattle with GT > 10 min of 66%, and an overall mortality of 34% in this study population. The overall mortality in septic cows was 46% and the mortality in non-septic cows was 31%.

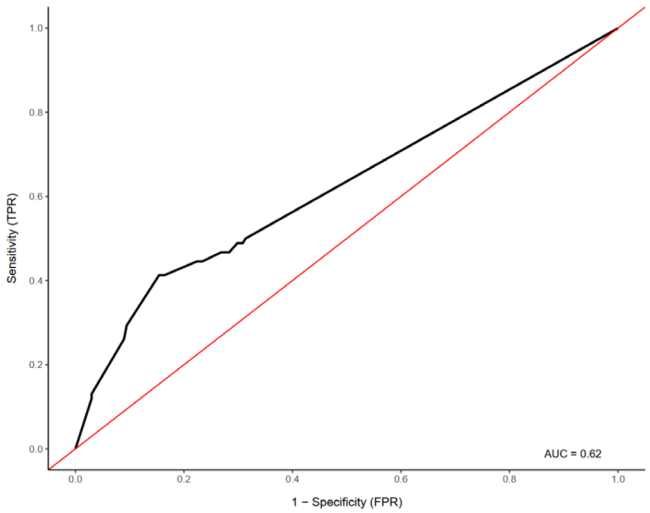

FIGURE 2 | Receiver operating characteristic (ROC) curve including only cows without sepsis ($n = 293$) for prediction of short-term death. The area under the curve is 0.62 (95% CI, 0.52–0.65). The point furthest from the diagonal line in a perpendicular sense shows the sensitivity and 1-specificity values that define our threshold for the GT, which would be a threshold of ≤ 3 min (sensitivity 0.41; 1-specificity 0.15; Youden Index 0.26). AUC, area under the curve; TPR, true positive rate; FPR, false positive rate.

7 | Long-Term Death

Overall, 170 cattle (71%) survived and 71 (29%) died during the 1-year follow-up period after discharge (Table 1). The study population in the long-term period included only 241 cattle because cattle that died during hospitalization are missing. Of the 241 included cattle, 170 (71%) had GT results ≥ 10 min and only 35 cattle (15%) had GT results ≤ 3 min.

8 | Association Between GT and Long-Term Death

Overall, cattle with GT results ≤ 3 min were not significantly more likely to die long-term based on LHR (Table 2), and no differences were found between non-septic or septic cattle. Based on these results, we did not calculate diagnostic test characteristics for this outcome or construct logistic regression models.

9 | Discussion

In our study, the GT was found to be a specific but less sensitive test to predict short-term death in adult cattle. The correlation of the GT with fibrinogen and TP was high for cattle without signs of sepsis. The prognostic ability of the GT in the short term was markedly influenced by the presence of sepsis, which aligns with our hypothesis.

Our hypothesis was based on previous studies showing that the positive predictive values of the GT for the prognosis of death for cattle with an inflammatory process are 81.8% (1 min) and 73.0% (3 min) with a prevalence of 69% [5]. Most studies reported a good predictive value of death using the GT for cattle with chronic diagnoses [2, 5, 6]. Based on our results, the GT is helpful to predict short-term death for cattle without sepsis but is not a reliable diagnostic test in cattle with acute sepsis. This finding once again emphasizes the importance of a thorough physical examination and the need to critically assess patient selection for diagnostic tests.

In contrast to previous studies, our PPV was low because many cattle with GT ≤ 3 min, which we would expect to have a poor prognosis, survived. This result could be because these cattle were accurately diagnosed and treated in a timely manner, or because cattle in Switzerland have a higher emotional and economic value than in many other places. The latter leads to a lower likelihood of owners electing to euthanize or slaughter their cattle based on a guarded prognosis, whereas in a previous study with higher GT PPV, the authors documented that many cattle that were defined as not cured were slaughtered because of economic reasons, which could have heavily impacted the results of the PPV [5]. Another factor to consider is that a shortened GT alone does not provide any information about the type and localization of the inflammatory process, which can substantially influence the prognosis of the patient.

TABLE 4 | Results of logistic regression model for the prediction of short-term death including clinically and biologically relevant variables with $p < 0.1$ from univariate analysis using a forward stepwise selection ($n = 358$).

Short-term death						
Variable	**Number of cattle**	**Odds ratio**	**95% CI**	**Coefficient**	**Standard error**	***p***
GT						
> 3 min	285	4.54	2.52–8.20	Ref	0.30	< 0.001
≤ 3 min	73			1.51		
Band neutrophiles						
No	148	2.36	1.59–3.50	Ref	0.20	< 0.001
Yes	210			0.86		
Antimicrobial at home or in clinic						
No	75	0.15	0.08–0.26	Ref	0.30	< 0.001
Yes	283			−1.93		

Note: GT was divided in 2 categories based on the cut-off value determined with the ROC curve: results ≤ 3 min, > 3 min. The following variables were offered to the multivariable regression model based on univariable screening and biological relevance: fever in hospital, use of an antimicrobial, band neutrophils, sepsis, lactate and GT.

TABLE 5 | Results of decreased logistic regression model predicting short-term death for cattle with and without sepsis.

Short-term death						
Variable	**Number of cattle**	**Odds ratio**	**95% CI**	**Coefficient**	**Standard error**	***p***
Non-septic cows						
GT						
> 3 min	224			Ref		
≤ 3 min	69	3.86	2.19–6.79	1.35	0.29	< 0.001
Septic cows						
GT						
> 3 min	68			Ref		
≤ 3 min	6	0.56	0.10–3.28	−0.58	0.90	0.52

Note: GT was divided into 2 categories as follows based on the cut-off value determined with the ROC curve: results ≤ 3 min, > 3 min. GT > 3 min was the referent category.

Because the aim of our study was to analyze the outcome of cattle with different GT results, we included all cattle admitted to the hospital for internal medicine reasons, not considering if cattle were diagnosed with an inflammatory disease or not. Many of the included cattle presented for non-inflammatory conditions, including abomasal or intestinal surgical emergencies. This factor led to a large number of cattle in our study with a negative GT (≥ 10 min) and a much smaller number of cows with GT ≤ 3 min. Although this design aspect may have influenced our diagnostic test characteristics, including the strength of our correlation coefficients, we elected to present the data as is to provide an accurate assessment of a real-life scenario practitioners may face.

One also must consider the substantial influence of disease prevalence on PPV and NPV. The disease prevalence in our study relates to the proportion of cows with a shortened GT result (≤ 3 min) in relation to the total number of cows in the study population. Our relatively low prevalence of cattle with GT ≤ 3 min resulted in low PPV and higher NPV. Although we feel that the distribution of GT results may accurately depict populations of hospitalized cattle elsewhere, caution must be exercised when extrapolating our results to populations with a different prevalence of low GT results.

Our PPV and NPV likely would be different if we had selected for cows with confirmed inflammatory disease. The PPV was lower in cattle with sepsis than in those without sepsis, which supports our hypothesis that the GT is not useful in predicting death in septic cattle.

Cattle with a GT ≥ 10 min in our study were less likely to die during hospitalization. Similar findings were reported in a previous study [2], but there are important limitations for cattle with GT that do not coagulate. Acute inflammatory disease and sepsis as well as perforated lesions such as abomasal ulcers can lead to loss of fibrinogen and other proteins into the abdominal cavity, which would make the GT unreliable in these disease processes [35].

The GT was not a helpful predictor for long-term outcome in our study. This result can be attributed to difficulties in predicting other factors (e.g., other diseases or financial issues of owners during the follow-up period). When talking to the owners on the phone and asking them why their cattle died, most of the cattle were slaughtered because of poor reproductive performance. Infertility can be caused by multiple underlying conditions that are not necessarily associated with a shortened GT or inflammatory disease.

Many of the cattle in our study were presented to the hospital with non-inflammatory diseases such as LDA that were surgically corrected. However, some of these cattle still had negative long-term outcomes. Unfortunately, it is impossible to know whether the reasons for slaughter at home were associated with the disease that was diagnosed at the hospital.

We hypothesized that sepsis would lead to a higher risk of death and that the GT and its prognostic potential may be heavily influenced by the presence of sepsis. The GT is often longer (≥ 10 min) in septic cattle or if the blood does not coagulate. Sepsis is a life-threatening emergency that occurs when the body's response to infection damages vital organs and can ultimately cause death. For clinical decision-making, it is important to have reliable and rapid tests to identify septic patients. Clinical examination findings can indicate the presence of sepsis in the field setting. These include injected episcleral vessels, hypo- or hyperthermia, tachycardia, tachypnea, hyperemia of the mucous membranes, decreased capillary refill time and dehydration [7, 36–38]. Blood cultures are needed to confirm sepsis, but take 48–72 h for results to become available and have several other limitations [39]. Sepsis also can be defined by the presence of toxic changes in the morphology of neutrophils, which can be observed in association with inflammatory diseases [22, 23]. The term toxic is not entirely correct because the cells have normal function, but they contain increased numbers of certain organelles that are usually formed during early development of neutrophils (e.g., toxic granules, Döhle bodies and cytoplasmic vacuolation) [24–26]. Based on our results, the GT was not useful as a prognostic marker in septic cattle. This finding emphasizes the importance of identifying animals with sepsis and acknowledges that using the GT as a prognostic marker in these patients is not appropriate for predicting outcome.

Band neutrophil count and use of antimicrobials also were predictive of mortality in our study. Increased band neutrophils can be seen in cattle with severe inflammatory processes and therefore can be associated with poor prognosis [26]. Use of antimicrobials should be limited to patients with suspicion of bacterial infection and although our results show an increased probability of survival when cattle received antimicrobials at home or in the hospital, the decision of choosing the right medication for treatment is more complex. Most of the cattle with GT results ≤ 3 min had diagnoses that usually are associated with chronic inflammatory processes and in these cattle treatment with antibiotics was highly recommended, and the use of antimicrobials likely increased their chance of survival.

Establishing a GT cut-off to predict mortality in cattle has long been attempted to optimize prompt medical decision-making. A previous study proposed a GT cut-off at a coagulation time of 7.1 min in cattle, where the maximal efficiency for detection of increased gamma globulin and fibrinogen concentrations was achieved [40]. We identified a GT threshold of ≤ 3 min to be indicative of a higher risk of death, whereas GT results of 3.5–9.5 min were not helpful in predicting outcome (Table 2). A GT coagulation time of ≥ 10 min was indicative of a decreased risk of death in our study. Previous studies suggested a coagulation time of 15 min as the cut-off for a negative GT result [2]. One study determined a cut-off of 14 min for prediction of an inflammatory state based on clinical findings and haptoglobin and serum amyloid A concentrations [41].

In agreement with previous studies [4, 6], GT results had a strong negative correlation with fibrinogen and serum total protein concentrations, particularly in non-septic cattle in our study population. A recently published study detected similar correlation coefficients in hospitalized cows with a GT ≤ 15 min and plasma serum protein ($r = -0.61$, $p < 0.001$) and plasma acute phase proteins such as fibrinogen ($r = -0.7$, $p < 0.001$) [41].

Fibrinogen and total protein can be helpful markers in detecting inflammatory processes in cattle. Although total protein can be assessed using a handheld portable refractometer, it still requires centrifugation of the blood sample before analysis, which is challenging to do on the farm. Fibrinogen measurement usually is only available in hospital and laboratory settings. Hence, the GT could serve as a practical on-farm alternative to estimate these variables.

Limitations of our study include its retrospective design and the potential variation and inaccurate or missing data that can come with this study design. The study population was limited to cattle that presented to the internal medicine service of the hospital on a referral-only basis, which are often much sicker than patients that present for orthopedic or reproductive issues or patients seen in the field. The exact time of coagulation whenever the GT was above 10 min was not recorded, which led to an inability to determine how many samples did not coagulate at all (as can be seen in cattle with generalized peritonitis). Furthermore, the information for our study was collected in a teaching hospital where the GT was performed by a variety of different people (mostly students or veterinary technicians), which could have led to inconsistencies in recording. On the other hand, the use of the GT by different people increases the robustness of our test results because it is also performed by different people on the farm. Lastly, the GT in our study was only performed once (on admission) and it is unknown if repeated GT performance would improve prognostic ability for survival or death.

In summary, the GT is useful for predicting chronic inflammation because of its correlation with globulins and fibrinogen. It also can be helpful for predicting death in cattle > 1 year of age without sepsis when considering accompanying clinical findings. The GT should not be used as a prognostic indicator in septic cattle, and hence, a thorough physical examination always should precede GT performance. The prognosis for non-septic cattle with a GT ≤ 3 min remains guarded, particularly in cattle with severe, chronic inflammatory disease, whereas non-septic cattle with a GT ≥ 10 min had a better prognosis for survival, especially in the short term. The use of rapid, inexpensive, and accurate point-of-care diagnostic tests is necessary to provide

appropriate care for animals that have a high chance of recovery and to avoid treatment and pain for those animals that likely will die.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Authors declare no institutional animal care and use committee or other approval was needed. Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.