Journal of Veterinary Internal Medicine

Journal of Veterinary Internal Medicine Open Access
ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE OPEN ACCESS

Equine Infectious Disease

The Systemic Inflammatory Response Syndrome and Predictors of Infection and Mortality in 1068 Critically Ill Newborn Foals

Pamela A. Wilkins[1] | David Wong[2] | Nathan M. Slovis[3] | Niamh Collins[4] | Bonnie S. Barr[5] | Catriona MacKenzie[6] | Cristobal Navas De Solis[7] | Carolina Castagnetti[8] | Jole Mariella[8] | Teresa Burns[9] | Gillian Perkins[10] | Barbara Delvescovo[10] | L. Chris Sanchez[11] | Ann M. Kemper[1] | K. Gary Magdesian[12] | Daniela Bedenice[13] | Sandra D. Taylor[14] | Jenifer Gold[15] | Bettina Dunkel[16] | Gene Pranzo[17] | Peter D. Constable[1]

[1]College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Ubana, Illinois, USA | [2]Veterinary Clinical Sciences, Lloyd Veterinary Medical Center, Iowa State University, Ames, Iowa, USA | [3]Hagyard Equine Medical Institute, Lexington, Kentucky, USA | [4]Scone Equine Hospital, Scone, New South Wales, United Kingdom of Great Britain and Northern Ireland | [5]Rood and Riddle Equine Hospital, Lexington, Kentucky, USA | [6]Rossdales Equine Hospital, Newmarket, United Kingdom of Great Britain and Northern Ireland | [7]Texas A&M, College Station, Texas, USA | [8]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy | [9]Veterinary Clinical Sciences, The Ohio State University, Columbus, Ohio, USA | [10]Department of Clinical Sciences, Cornell University, Ithaca, New York, USA | [11]Large Animal Clinical Sciences, University of Florida, Gainesville, Florida, USA | [12]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA | [13]Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts, USA | [14]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, Indiana, USA | [15]Washington State University, Pullman, Washington, USA | [16]Veterinary Basic Sciences, Royal Veterinary College, North Mymms, Hertsfordshire, United Kingdom of Great Britain and Northern Ireland | [17]Dorothy Russell Havemeyer Foundation, New York, USA

Correspondence: Peter D. Constable (constabl@illinois.edu)

Received: 22 August 2024 | **Revised:** 29 December 2024 | **Accepted:** 14 January 2025

Funding: The authors received no specific funding for this work.

Keywords: bacteremia | hyperlactatemia | sepsis | shock

ABSTRACT

Background: Sepsis has been defined in humans as the concurrent proven or suspected presence of microbial infection and the systemic inflammatory response syndrome (SIRS). Sepsis is the leading cause of morbidity and mortality in neonatal foals. The clinical utility of using SIRS or its individual components to predict infection and mortality in critically ill foals is currently unknown.

Objectives: Assess the ability of history and signalment, clinical findings, laboratory results, and SIRS-related indices to predict infection and mortality in critically ill foals.

Animals: Retrospective, multi-center, cross-sectional study using a convenience sample of 1068 critically ill foals < 3 days of age admitted to 16 veterinary referral hospitals in 4 countries.

Methods: Data were retrieved from medical records. Infection was defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus on admission. Univariate non-parametric and categorical methods, multivariate logistic regression, and classification tree methods were used for statistical analysis.

Abbreviations: AG, anion gap; AUC, area under the curve; ROC, receiving operating characteristic; SID_4, measured strong ion difference; SIG, strong ion gap; SIRS, systemic inflammatory response syndrome; USI, unmeasured strong ions.

Results: Foal age at admission and presence of toxic neutrophils were independent predictors of infection, whereas SIRS-related indices were not predictive of infection. In-hospital mortality was 24%. Independent predictors for mortality were hypokinetic pulses, cold extremities, presence of seizures, blood L-lactate concentration > 6.0 mmol/L, and increased serum potassium and total bilirubin concentrations.

Conclusions and Clinical Importance: The presence of infection in critically ill newborn foals was not predicted by SIRS indices. Cardiovascular dysfunction was strongly associated with mortality, suggesting that maintaining adequate perfusion and pulse pressure should be important treatment goals.

1 | Introduction

The systemic inflammatory response syndrome (SIRS) is a generalized inflammatory state that is identified using selected physical examination findings and the results of laboratory analysis [1, 2]. Sepsis in foals has been defined as the concurrent identified or suspected presence of microbial infection and SIRS,[a] and is a common cause of morbidity and mortality in newborn and neonatal foals [1–3]. Treatment of sepsis requires an intensive approach that includes prompt administration of antimicrobial drugs, IV administration of fluids and vasopressor agents to ensure adequate oxygen delivery and mean arterial blood pressure, ancillary treatments for inflammation and coagulopathy, and frequent monitoring of organ function [3, 4]. Sepsis in newborn foals frequently is accompanied by failure of transfer of passive immunity, and newborn foals with clinical signs of omphalophlebitis, meningoencephalitis, arthritis, pneumonia, or enterocolitis often are assumed to have sepsis [4]. Isolation of bacteria from a blood culture without concurrent presence of systemic inflammation does not meet the current definition of sepsis in foals[a].

The definition of SIRS in adult humans originally was based on the presence of 2 or more of the following: abnormal temperature, tachycardia, tachypnea, or abnormal leukocyte count [5]. Studies in calves [6], dogs, cats [7], and foals [8–10], have employed SIRS-related indices with species and age adjusted cutpoints for the reference range, but, to our knowledge, the clinical utility of the SIRS construct in domestic animals has not been proven. We hypothesized that infection and the likelihood of mortality in newborn foals could be predicted on admission to veterinary referral hospitals, based on history, signalment, physical examination findings, laboratory results, and SIRS-related indices. With this information, we intended to develop a definition of SIRS in newborn foals that would be validated using prospectively collected data.

2 | Materials and Methods

2.1 | Animals

The study population was a convenience sample of newborn foals ($n = 1068$) ≤ 3 days of age admitted to 16 veterinary referral hospitals in 2016 and 2017 (Table S1). Thirteen of the hospitals were in the United States, with 1 each in Australia, England, and Italy. At least 40 breeds of horses were represented, with Thoroughbreds, Quarter Horses, and Standardbreds accounting for 603 (56%) of the study population (Table S2). Ponies and other Equidae were excluded from the study. Newborn colt foals comprised 59% of total newborn admissions.

2.2 | Review of Medical Records and Calculation of SIRS Indices

Information retrieved from the medical records included signalment (age, sex, breed), dam age and parity, whether the current and previous parturitions were normal or abnormal, if the foal was premature based on its gestation length, whether the dam had produced an abnormal foal in previous pregnancies, and the duration of hospitalization. Medical record data was anonymized and approval to analyze the data in the medical record was obtained. The following physical examination findings were recorded for each foal at admission, when available: rectal temperature, heart rate, arterial pulse characteristics (normal or hypokinetic if the pulse was present but pulse pressure was decreased), respiratory rate, muscle tone (normal or decreased), mental state (normal or depressed), and the presence or absence of seizures, petechiae, musculoskeletal deformity, ≥ 1 infected sites, and cold extremities. Results from the following laboratory analyses were recorded for each foal at admission, when available: venous blood culture, hematologic analysis, serum biochemical analysis, and venous or arterial blood gas analysis. Blood cultures were obtained from foals by aseptic preparation of the skin over the jugular or cephalic vein, aseptic collection of at least 5 mL of blood through a needle or IV catheter into a blood culture tube and immediate aerobic culture at 37°C for at least 24 h. Larger collection volumes and additional culture methods were used at some hospitals.

Blood culture results were recorded as positive (≥ 1 bacterial species isolated) or negative, but information related to bacterial identification was not retrieved from the medical record. Blood pO_2 results were not recorded because of insufficient information in most records as to whether a venous or arterial sample had been obtained and whether the foal was receiving intranasal O_2 or other respiratory support at the time of sampling. Actual bicarbonate concentration ($cHCO_3^-$), base excess, and anion gap (AG) were calculated as described [6]. The simplified quantitative physicochemical strong ion approach [11, 12] also was used to allow a more comprehensive assessment of the acid–base status of the foals. Measured strong ion difference (SID_4, mmol/L) was calculated from the plasma concentrations of 4 strong ions (sodium, chloride, potassium, L-Lactate) such that:

$$SID_4 = cNa^+ + cK^+ - cCl^- - c(\text{L-lactate}^-) \quad (1)$$

An estimate of the unmeasured strong ion concentration was obtained by calculating the strong ion gap (SIG, mmol/L), defined as the difference between the plasma concentration of unmeasured strong cations and unmeasured strong anions [12], such that:

$$SIG = \left\{2.24 \times (\text{total protein}, g/dL)/\left(1+10^{(6.65-pH)}\right)\right\} - AG \quad (2)$$

Strong ion gap was corrected for measured plasma L-lactate concentration to obtain an estimate of the concentration of unidentified strong ions (USI, mmol/L) which represented the unmeasured strong ion difference (SID_{um}, mmol/L), such that:

$$USI = SID_{um} = SIG + c(L - \text{lactate}^{-}) \quad (3)$$

A diagnosis of SIRS was made on admission using recommendations from the 2018 Dorothy Russel Havemeyer SIRS and Sepsis in Foals Working Group.[a] For newborn foals, SIRS was defined as being present if 2 or more of the following criteria were fulfilled on admission: presence of an abnormal leukocyte count (i.e., leukopenia or leukocytosis; reference interval, 7000 to 14400 cells/μL, or >5% band neutrophils), abnormal rectal temperature (reference interval, 99.0 to 102.5°F), abnormal heart rate (i.e., tachycardia or bradycardia; reference interval, 60 to 115 beats per minute), abnormal respiratory rate (reference interval, 30 to 56 breaths per minute), provided that at least 1 of the criteria was abnormal rectal temperature or abnormal leukocyte count. A modified sepsis score for critically ill foals, as proposed previously [13], was not calculated because 1 or more components were not available for most foals.

2.3 | Statistical Analyses

The final data set used for analysis was developed by aggregating 16 separate data sets and standardizing the units for continuous variables and responses for categorical variables. Data entry errors were identified when physiologically implausible (e.g., cNa^+ of 4.1 mmol/L) and assigned as missing data. Software programs (PROC MIXED, PROC REG; SAS 9.4, SAS Inc., Cary NC; MedCalc Statistical Software version 15.11.4, MedCalc Software bvba, Ostend, Belgium; and the *rpart* package in R) were used for statistical analyses. A $p < 0.01$ was considered significant because of the large numbers of foals in the dataset and the number of predictors evaluated.

The primary purpose of our retrospective study was to identify variables that were predictive of the presence of infection and in-hospital mortality, with the intention being that highly predictive variables would be studied in more detail in a future prospective multi-center study. As such, the data set was not separated into training and test samples. The initial statistical analysis was confined to identifying predictors and associations for newborn foals on admission. Newborn foals were defined as those ≤72 h of age. This age range was selected for analysis because of the distinct age-related and maturational physiological differences over the first few weeks of life in foals [14],[a] and age-specific ranges for diagnosing SIRS have been recommended for use in humans [15]. Categorical data were presented as percentages and proportions and compared using the Chi-square test. Continuous data were presented as medians and ranges because most of the data were not normally distributed as indicated by the Shapiro–Wilk W test and visual examination of QQ-plots. The Mann Whitney U-test was used for comparison of continuous variables.

The first outcome variable of interest was the identification of infection, defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus on admission. The study population for this analysis was newborn foals that had at least 1 blood culture performed on admission to 16 veterinary referral hospitals in 2016 and 2017. The second outcome variable of interest was in-hospital mortality, defined as newborn foals that died or were euthanized during their hospital stay. The study population for this analysis was all newborn foals admitted to 16 veterinary referral hospitals in 2016 and 2017. Binomial logistic regression and classification tree methods were used to characterize the associations between various factors and the presence of bacteremia or an infected focus on admission and in-hospital mortality.

A stepwise forward binomial logistic regression model was developed to determine the relationship between in-hospital mortality and selected independent categorical or continuous variables of interest that had a p value for predicting infection or mortality < 0.05 for entry into the model. Based on the study factors of interest, 3 logistic regression models were built: the first model used all potentially useful predictors that could be obtained rapidly before or on admission (history, signalment, and physical examination model); the second model was based exclusively on laboratory findings (laboratory model); and the third model utilized all available data. Variables were selected for inclusion in the stepwise logistic regression procedure if they were biologically relevant based on evidence of being predictive of outcome in previous studies. Moreover, to minimize the effects of collinearity, when 2 continuous variables were closely correlated ($r_s > |0.60|$), only the variable that had the lowest p value for the outcome of interest was entered into the model. The relative importance of included variables was assessed by the order of entry into the model. Interaction terms were not investigated for each significant predictor in the multivariate model because interaction terms make it difficult to interpret coefficient values [16]. The fit of each final logistic regression model was evaluated by means of the Hosmer-Lemeshow goodness-of-fit test. The area under the receiver operating characteristic (ROC) curve was calculated and used as a global index of test performance. Area under the curve (AUC) values for ROC curves >0.9 typically indicate a highly accurate test, whereas AUC values of 0.7–0.9 indicate moderate accuracy, 0.5–0.7 low accuracy, and 0.5 a chance result [17]. Sensitivity (Se) and specificity (Sp) were calculated at the optimal cut-point of the ROC determined by the Youden index (the cut-point where the following expression has its maximum value: Se + Sp = 1), which equally weights Se and Sp. The overall accuracy (percent of correct predictions) also was calculated.

Classification tree analysis was performed using surrogate variables for foals with missing data for 1 or more factors. The Gini splitting criterion was used to initially identify the variable and split pattern that optimally partitioned the node, provided that each node contained >10% of the study population. The fitted

tree then was pruned by using the complexity parameter value associated with the lowest cross-validated error obtained from 10-fold cross validation. The latter method minimizes overfitting of the model by randomly splitting the data set into 10 parts and averages the results for 10 classification trees obtained by leaving out 1 of the 10 parts in turn.

3 | Results

3.1 | Foals With an Identified Infection

Blood cultures were obtained from 496 foals (46.4% of 1068 foals), with 134 (27.0%) being positive (Table 1). The presence or absence of infections at non-blood sites was stated in the medical records of 605 foals, of which 455 foals had a blood culture performed. Forty-five of the 455 foals (9.9%) had an infection identified at a non-blood site by physical examination or laboratory methods such as cytology or culture of an aspirate or tissue sample; 15 of these foals were also bacteremic. The data set that was analyzed therefore contained 455 newborn foals with an infection identified ($n = 164 = 134 + 30$; 36.0%) or no infection detected ($n = 291$; 64.0%). Table 2 contains SIRS-related indices, signalment, history, physical examination findings and laboratory results of foals with and without an identified infection.

The 2018 Havemeyer definition for SIRS[a] could be determined for 431 foals, which represented 94.7% of the study population of foals with or without an identified infection. The overall prevalence of SIRS was high (69.6%), and the percentage of foals with an infection identified on admission that had SIRS (73.1%) was not different from the percentage of foals that did not appear to have an infection (71.3%). Univariate analysis indicated that foals with an identified infection were older on admission (Table 2). Most of the significant differences were for laboratory results. Foals with an identified infection at admission had decreased leukocyte, neutrophil, lymphocyte, and monocyte counts, and a higher proportion of foals had band neutrophils and toxic neutrophils present. Foals with an identified infection had lower serum GGT activity and serum urea nitrogen concentration and higher fibrinogen concentration on admission.

Multivariate logistic regression using signalment, history, and physical examination data available for > 50% of the foals (> 227) identified foal age as a predictor for identified infection (Table 3). The ROC curve for the model had an AUC of 0.62 (Figure 1, left panel). The predicted probability of infection increased almost linearly with time since birth (Figure 1, right panel). Multivariate logistic regression using laboratory data that was available for > 50% of the foals and based on 12 potential predictors with $p < 0.05$ ($n = 270$) identified toxic neutrophils as a predictor for identified infection (Table 3). The ROC curve for the model had an AUC of 0.64 (Figure 2) and Se = 0.57 and Sp = 0.68. Multivariate logistic regression using all data available for > 50% of the foals and based on 13 potential predictors identified 2 predictors for identified infection: foal age and presence of toxic neutrophils (Table 3). The ROC curve for the final 2 factor model had an AUC of 0.81 (Figure 3). Classification tree analysis for identified infection on admission based on all data available from 455 foals and the 13 predictor variables used for logistic regression (270 foals) did not produce a reliable predictive model.

3.2 | In-Hospital Mortality

The number of bacteremic and infected foals, SIRS-related indices, signalment and history, physical examination findings, and laboratory results on admission for survivors and non-survivors are summarized in Table 1. In-hospital mortality rate was 23.8%, with 814 foals surviving and 254 foals not surviving until discharge. Of the 254 foals, 86 (33.9%) died spontaneously, whereas 168 (66.1%) were euthanized.

The number of foals in different clinical categories and the respective proportion of non-surviving foals are summarized in Table 1. Blood cultures were obtained at admission in 46.4% of the foals and were obtained in a higher proportion in foals that did not survive (61.4%) than foals that survived (41.8%). The percentage of blood cultures that were positive for survivors (29.4%) was similar to that for foals that did not survive (21.8%).

The 2018 Havemeyer definition for SIRS[a] could be determined for 653 foals, representing 61.1% of the study population. The overall prevalence of SIRS was high (64.9%), and the prevalence of SIRS was higher in non-survivors (81.3%) than survivors (60.5%). The 2018 Havemeyer definition of SIRS[a] was predictive of in-hospital mortality in the univariate analysis. Chi-square analysis indicated that of the four 2018 Havemeyer SIRS-related indices[a], abnormal rectal temperature and leukocyte count were predictive of in-hospital mortality, whereas abnormal heart rate or respiratory rate were not predictive of outcome.

Univariate analysis indicated that rectal temperature was lower, capillary refill time longer, and foal age at admission lower in non-survivors (Table 1). Chi-square analysis indicated that abnormal parturition, prematurity of the foal, and presence of decreased muscle tone, depressed mental status, seizures, cold extremities, hypokinetic pulses, hyperemic mucous membranes, petechiae, musculoskeletal deformity, and an infected site other than the intravascular compartment (e. g., septic arthritis, omphalophlebitis, pneumonia, uveitis) were predictive of in-hospital mortality.

Univariate analysis identified significant differences between survivors and non-survivors for many clinical pathology analytes (Table 1). Compared with survivors, non-survivors had lower blood pH, $cHCO_3^-$, and base excess, and higher blood L-lactate concentration and AG on admission. Unmeasured strong ion concentration (SID_{um}) was similar and low for survivors (−2.0 mmol/L) and non-survivors (−2.3 mmol/L). Total protein and IgG concentrations were lower in non-survivors, whereas albumin concentrations were similar. Non-survivors also had lower glucose concentrations, higher potassium, phosphate, and creatinine concentrations, and higher creatine kinase activity than survivors on admission. Consistent differences in leukocyte numbers were observed between the 2 groups, with non-survivors having lower numbers of leukocytes, neutrophils, monocytes, and platelets than survivors on admission, as well as lower neutrophil to lymphocyte ratios. Despite lower overall

TABLE 1 | Association of in-hospital mortality with identification of infection, SIRS-related indices, signalment and history, physical examination findings, and laboratory values on admission of 1068 newborn foals < 3 days of age.

Identification of infection	*N*	*n*	Survivors number (%)	*n*	Non-survivors number (%)	*p*
Blood culture performed (% total)	496	340	41.8% (340/814)	156	61.4% (156/254)	**< 0.0001**
Blood culture positive (% cultured)	134	100	29.4% (100/340)	34	21.8% (34/156)	0.08
Non-blood infected site identified	605	483	6.0% (29/483)	122	13.1% (16/122)	**0.01**
Infection identified	455	349	34.1% (119/349)	106	42.5% (45/106)	0.12

SIRS-related indices	*N*	*n*	Survivors number (%)	*n*	Non-survivors number (%)	*p*
Abnormal temperature (%)	988	776	21.7% (168/776)	212	43.9% (93/212)	**< 0.0001**
Abnormal heart rate (%)	1007	788	42.3% (333/788)	219	51.1% (112/219)	0.02
Abnormal respiratory rate (%)	987	776	48.1% (373/776)	211	54.5% (115/211)	0.10
Abnormal leukocyte count (%)	693	541	56.4% (305/541)	152	73.0% (111/152)	**0.0002**
SIRS present–2018 Havemeyer definition (%)	653	514	62.5% (321/514)	139	81.3% (113/139)	**< 0.0001**

Signalment and history	*N*	*n*	Survivors % or median and (range)	*n*	Non-survivors % or median and (range)	*p*
Parturition abnormal	891	682	36.7% (250/682)	209	54.1% (113/209)	**< 0.0001**
Premature foal	874	677	5.9% (40/677)	197	15.2% (30/197)	**< 0.0001**
Gestation length (days)	696	537	340 (281–425)	159	340 (298–402)	0.01
Foal age (hours)	970	742	12 (0–72)	228	5 (0–72)	**0.0002**
Dam age (years)	588	458	9 (3–25)	130	10 (4–23)	0.62
Dam parity	455	356	2 (1–11)	99	2 (1–13)	0.45
Dam had abnormal foal previously	458	363	25.1% (91/363)	95	27.4% (26/95)	0.65
Filly foal	986	770	40.7% (313/770)	216	39.4% (85/216)	0.73
Placed on intranasal O_2	582	457	21.1% (96/457)	125	60.8% (76/125)	**< 0.0001**
Duration of hospitalization (days)	1038	798	5 (1–92)	240	2 (0–40)	**< 0.0001**

Physical examination findings	*N*	*n*	Survivors % or median and (range)	*n*	Non-survivors % or median and range	*p*
Rectal temperature (°F)	988	776	100.5 (91.9–105.8)	212	99.6 (90.2–104.8)	**< 0.0001**
Heart rate (beats/min)	1007	788	104 (50–205)	219	106 (32–200)	0.28
Respiratory rate (breaths/min)	987	776	32 (4–140)	211	32 (4–120)	0.88
Decreased muscle tone	725	564	31.0% (175/564)	161	66.5% (107/161)	**< 0.0001**
Depressed	736	559	30.2% (171/569)	167	63.5% (106/167)	**< 0.0001**
Seizures present	1023	804	3.9% (31/804)	219	18.7% (41/219)	**< 0.0001**
Cold extremities	968	762	19.4% (148/762)	206	54.9% (113/206)	**< 0.0001**
Hypokinetic pulses	962	755	14.0% (106/755)	207	50.2% (104/207)	**< 0.0001**
Hyperemic mucous membranes	938	741	19.2% (142/741)	197	41.1% (81/197)	**< 0.0001**

(Continues)

TABLE 1 | (Continued)

Physical examination findings	*N*	*n*	Survivors % or median and (range)	*n*	Non-survivors % or median and range	*p*
Capillary refill time (seconds)	303	243	2 (1–4)	60	2 (1–5)	**0.001**
Petechiae	996	781	3.1% (24/781)	215	8.4% (18/215)	**0.01**
Musculoskeletal deformity	1000	779	15.2% (118/779)	221	24.9% (55/221)	**0.01**
Laboratory values	***N***	***n***	**Survivors median (range)**	***n***	**Non-survivors median (range)**	***p***
Venous blood pH	196	138	7.38 (6.86–7.50)	58	7.30 (6.90–7.48)	**<0.0001**
pCO_2 (mm Hg)	302	224	39 (12–113)	78	45 (16–134)	0.04
HCO_3 (mmol/L)	192	135	26.8 (10.8–42.1)	57	23.6 (10.8–37.1)	**0.01**
Base excess (mmol/L)	192	135	1.8 (−15.9–17.5)	57	−2.6 (−17.8–10.7)	**0.01**
Na (mmol/L)	770	609	137 (115–165)	161	137 (112–154)	0.63
K (mmol/L)	722	557	3.8 (1.1–11.8)	165	4.2 (2.0–7.5)	**<0.0001**
Total Ca (mg/dL)	685	535	11.2 (3.9–19.7)	150	11.5 (6.4–20.2)	0.04
Ca-ionized (mmol/L)	188	141	1.46 (0.73–2.25)	47	1.48 (0.81–2.20)	0.12
Mg-ionized (mmol/L)	187	148	0.68 (0.22–1.19)	39	0.58 (0.24–1.20)	0.08
Cl (mmol/L)	772	601	96 (69–123)	171	96 (71–124)	0.67
P (mg/dL)	624	491	5.4 (2.4–19.8)	133	5.8 (3.4–14.3)	**0.01**
L-lactate (mmol/L)	607	454	3.5 (0.6–23.0)	153	7.0 (0.9–23.0)	**<0.0001**
Anion gap (mmol/L)	217	128	15.1 (−9.0–46.6)	49	19.0 (6.8–29.0)	**0.01**
SID_4 (mmol/L)	537	404	39.8 (12.9–56.6)	133	37.4 (17.3–52.0)	**0.01**
Strong ion gap (mmol/L)	170	124	−6.0 (−41.4–17.5)	46	−8.7 (−21.5–2.9)	0.02
SID_{um} (mmol/L)	162	118	−2.0 (−19.6–20.3)	44	−2.3 (−13.8–8.0)	0.89
GGT (U/L)	410	321	29 (7–1347)	89	25 (7–79)	0.02
AST (U/L)	473	375	170 (39–2316)	98	173 (24–3476)	0.83
CK (U/L)	472	376	479 (27–55 020)	96	764 (41–22 000)	**0.003**
Glucose (mg/dL)	811	629	126 (10–306)	182	96 (3–375)	**<0.0001**
Total bilirubin (mg/dL)	696	551	3.7 (0.1–27.7)	145	4.0 (0.1–16.7)	0.01
Creatinine (mg/dL)	849	663	2.2 (0.5–31.9)	186	3.3 (0.7–28.8)	**<0.0001**
SUN (mg/dL)	657	520	25 (2–167)	137	33 (5–577)	0.01
PCV (vol %)	705	550	39 (10–54)	155	39 (8–70)	0.97
Total protein (g/dL)	926	732	4.8 (2.9–8.4)	194	4.4 (2.3–8.2)	**0.002**
Albumin (g/dL)	744	583	2.5 (1.4–4.3)	161	2.6 (1.4–3.6)	0.96
Globulin (g/dL)	724	566	2.2 (0.2–6.0)	158	1.9 (0.0–5.6)	0.02
IgG (mg/dL)	533	441	800 (2–5000)	92	498 (0–2333)	**<0.0001**
Serum amyloid A (mg/dL)	106	85	67 (0.5–1996)	21	242 (1.5–825)	0.28

(Continues)

TABLE 1 | (Continued)

Laboratory values	*N*	*n*	Survivors median (range)	*n*	Non-survivors median (range)	*p*
Fibrinogen (mg/dL)	614	488	300 (100–1200)	126	365 (50–1320)	0.08
Leukocytes (cells/μL)	693	541	7400 (780–94400)	152	4990 (550–23560)	**<0.0001**
Neutrophils (cells/μL)	666	526	5800 (70–84960)	140	3170 (20–18850)	**<0.0001**
Band neutrophils (cells/μL)	359	286	0 (0–10400)	73	0 (0–36000)	**<0.0001**
Band neutrophils (% of total)	359	285	0 (0–99.0)	73	0 (0–99.8)	**<0.0001**
Band neutrophils present	359	286	32.2% (92/286)	73	41.1% (30/73)	0.15
Toxic neutrophils present	477	382	33.8% (129/382)	95	49.5% (47/95)	**0.005**
Lymphocytes (cells/μL)	667	526	1287 (170–8500)	141	1170 (48–4600)	0.16
Neutrophil to lymphocyte ratio	662	523	4.6 (0.1–92.0)	139	2.4 (0.1–46.0)	**<0.0001**
Monocytes (cells/μL)	660	524	190 (0–1700)	136	108 (0–1890)	**0.0003**
Eosinophils (cells/μL)	656	523	0 (0–600)	133	0 (0–300)	0.88
Basophils (cells/μL)	642	511	0 (0–384)	130	0 (0–50)	0.21
Platelets (cells/μL)	347	274	190000 (4000–463000)	73	161000 (12000–420000)	**0.003**

Note: Data were obtained from the medical records of 16 referral veterinary hospitals over 2 years. The number of survivors and non-survivors were 814 (76.2%) and 254 (23.8%), respectively. *N* is the total number of foals with data available for the study variable; *n* is the number of foals with data available that survived or did not survive.
Abbreviations: AST = aspartate aminotransferase activity, CK = creatine kinase activity, GGT = gamma glutamyltransferase activity, IgG = Immunoglobulin G concentration, PCV = packed cell volume, SIRS = systemic inflammatory response syndrome, SUN = serum urea nitrogen. Variables and thier associated *p* values that are significantly ($p < 0.01$) associated with infection are presented in bold font.

neutrophil numbers, non-survivors had higher percentages of toxic neutrophils.

Multivariate logistic regression using signalment, history, and physical examination data available for > 50% of the foals (> 534) and based on 13 potential predictors with $p < 0.05$ identified 2 predictors for in-hospital mortality: hypokinetic pulses and cold extremities (Table 2). The ROC curve for the final 2-factor model had an AUC of 0.77 (Figure 3). Logistic regression using laboratory data that was available for > 50% of the foals and, based on 14 potential predictors with $p < 0.05$, identified 2 predictors for in-hospital mortality (Table 4): increased blood L-lactate concentration and increased plasma or serum potassium concentration (Table 2). The ROC curve for the final 2-factor model had an AUC of 0.76 (Figure 3). Logistic regression using all data that was available for > 50% of the foals and based on 27 potential predictors identified 2 predictors for in-hospital mortality: hypokinetic pulses and increased serum total bilirubin concentration (Table 2). The ROC curve for the final 2-factor logistic regression model had an AUC of 0.81 (Figure 3).

Classification tree analysis for observed foal mortality based on all data available is presented in Figure 4. The analysis indicated that in-hospital mortality was associated with 2 physical examination abnormalities (hypokinetic pulses, presence of seizures) and 1 laboratory abnormality (L-lactate concentration > 6.0 mmol/L). The most significant factor in predicting mortality was hypokinetic pulses, followed by the presence of seizures and increased blood L-lactate concentration. Overall diagnostic performance of the classification tree analysis was Se = 0.33, Sp = 0.93, and accuracy of 78.7%.

4 | Discussion

The driving force for our study was the urgent need to identify rapid, practical, and sufficiently accurate diagnostic tests for infection in newborn foals. Contrary to our hypothesis, we found that the ability to predict infection in critically ill newborn foals using history, signalment, physical examination findings, laboratory results, and SIRS-related indices was poor to fair. Indices related to SIRS, such as abnormal rectal temperature, heart rate, respiratory rate, and leukocyte count, were not predictive of infection in newborn foals.

Characterization of the leukocyte profile was the most informative predictor of the presence of infection. Categorical and non-parametric analyses indicated that the presence of toxic neutrophils; decreased leukocyte, neutrophil, lymphocyte, and monocyte counts; and, increased band neutrophil counts and plasma fibrinogen concentration were associated with the presence of infection. Logistic regression analysis indicated that the

TABLE 2 | Association of SIRS-related indices, signalment and history, physical examination findings, and laboratory values of 455 newborn foals without ($n = 291$, 64.0%) or with ($n = 164$, 36.0%) infection.

SIRS-related indices	*N*	*n*	No infection %	*n*	Infection %	*p*
Abnormal rectal temperature	445	283	29.7% (84/283)	162	30.9% (50/162)	0.79
Abnormal heart rate	447	284	37.7% (107/284)	163	36.1% (64/163)	0.74
Abnormal respiratory rate	442	280	52.9% (148/280)	162	59.3% (96/162)	0.19
Abnormal leukocyte count	442	284	64.4% (183/284)	158	69.0% (109/158)	0.33
SIRS present–2018 Havemeyer definition[a] (%)	431	275	72.4% (199/275)	156	75.6% (118/156)	0.46
Signalment and history	***N***	***n***	**No infection % or median and range**	***n***	**Infection % or median and range**	***p***
Parturition abnormal	365	243	45.7% (111/243)	122	40.2% (49/108)	0.32
Premature foal	359	231	11.3% (26/231)	128	6.3% (8/114)	0.12
Gestation length (days)	271	187	339 (311–425)	84	340 (300–361)	0.21
Foal age (hours)	406	259	9 (0–72)	147	24 (0–72)	**< 0.0001**
Dam age (years)	353	226	9 (3–24)	127	9 (3–25)	0.87
Dam parity	289	208	2 (1–13)	81	2 (1–11)	0.35
Previous abnormal foal	283	190	27.4% (52/190)	93	26.9% (25/93)	0.93
Filly foal	440	278	41.7% (116/278)	162	40.1% (65/162)	0.74
Physical examination findings	***N***	***n***	**No infection % or median and range**	***n***	**Infection % or median and range**	***p***
Rectal temperature (°F)	445	283	100.4 (91.9–105.8)	162	100.2 (92.8–104.8)	0.84
Heart rate (beats/min)	447	284	100 (50–200)	163	100 (40–200)	0.44
Respiratory rate (breaths/min)	442	280	32 (4–94)	162	32 (4–120)	0.27
Decreased muscle tone	440	286	44.4% (127/286)	154	45.5% (70/154)	0.83
Depressed	441	285	45.3% (129/285)	156	48.2% (69/156)	0.83
Seizures present	452	290	7.6% (22/290)	162	9.3% (15/162)	0.53
Cold extremities	433	285	32.6% (93/285)	145	39.2% (58/145)	0.17
Hypokinetic pulses	432	286	30.1% (86/286)	146	32.2% (47/146)	0.65
Hyperemic mucous membranes	398	248	40.3% (100/248)	150	39.3% (59/150)	0.85
Capillary refill time (seconds)	174	94	2 (1–4)	80	2 (1–5)	0.05
Petechiae	441	286	3.9% (11/286)	155	5.8% (9/155)	0.34
Musculoskeletal deformity	435	275	14.6% (40/275)	160	15.0% (24/160)	0.90
Placed on intranasal O_2	173	81	35.8% (29/81)	92	35.9% (33/92)	0.99
Laboratory values	***N***	***n***	**No infection % or median and range**	***n***	**Infection % or median and range**	***p***
Venous blood pH	122	67	7.36 (6.99–7.46)	55	7.34 (6.86–7.48)	0.53
pCO_2 (mm Hg)	126	68	49 (30–88)	58	45 (24–134)	0.21
HCO_3 (mmol/L)	122	67	25.9 (12.8–37.0)	55	24.4 (11.2–38.7)	0.11
Base excess (mmol/L)	122	67	1.4 (−15.3–10.9)	55	−0.6 (−15.9–16.3)	0.15
Na (mmol/L)	433	277	136 (115–165)	156	138 (117–156)	0.03

(Continues)

TABLE 2 | (Continued)

Laboratory values	*N*	*n*	No infection % or median and range	*n*	Infection % or median and range	*p*
K (mmol/L)	422	272	3.9 (2.0–7.5)	150	3.8 (1.1–6.4)	0.53
Total Ca (mg/dL)	407	266	11.3 (5.9–19.7)	141	11.2 (7.8–14.8)	0.02
Ca-Ionized (mmol/L)	96	48	1.48 (0.73–1.80)	48	1.45 (0.79–2.25)	0.13
Mg-Ionized (mmol/L)	107	60	0.44 (0.24–0.70)	47	0.42 (0.29–0.78)	0.40
Cl (mmol/L)	422	271	96 (69–123)	151	97 (77–124)	0.08
P (mg/dL)	394	260	5.4 (2.6–19.8)	134	5.8 (2.8–13.1)	0.14
L-lactate (mmol/L)	405	256	4.0 (0.8–23.0)	149	4.7 (0.6–21.7)	0.34
Anion Gap (mmol/L)	111	64	16.0 (2.1–39.0)	47	16.3 (5.4–34.6)	0.77
SID_4 (mmol/L)	367	236	39.2 (26.5–56.6)	131	38.6 (21–7 – 52.8)	0.31
Strong ion gap (mmol/L)	106	60	−6.2 (−41.4–6.8)	46	−7.4 (−23.5–3.0)	0.47
SID_{um} (mmol/L)	104	58	−1.5 (−19.6–8.0)	46	−2.7 (012.0–7.5)	0.55
GGT (U/L)	222	124	29 (7–88)	98	24 (7–1347)	**0.01**
AST (U/L)	223	124	195 (50–2110)	99	191 (33–3476)	0.77
CK (U/L)	234	136	581 (41–55 020)	98	535 (77–23 346)	0.87
Glucose (mg/dL)	427	266	116 (6–375)	161	121 (3–306)	0.93
Total Bilirubin (mg/dL)	414	278	3.6 (1.0–15.2)	136	4.3 (0.1–13.9)	0.01
Creatinine (mg/dL)	435	284	2.3 (0.5–30.2)	151	1.9 (0.7–30.3)	0.06
SUN (mg/dL)	384	244	38 (4–122)	140	28 (2–167)	**0.0003**
PCV (vol %)	445	285	39.0 (15.0–56.0)	160	38.5 (17–58)	0.65
Total Protein (g/dL)	438	281	4.7 (2.3–8.2)	157	5.0 (3.0–8.4)	0.02
Albumin (g/dL)	418	277	2.4 (1.4–4.3)	141	2.5 (1.4–3.8)	0.01
Globulin (g/dL)	410	270	2.2 (0–5.6)	140	2.2 (0.7–6.0)	0.26
IgG (mg/dL)	257	149	800 (0–3135)	108	708 (2–2813)	0.74
Serum Amyloid A (mg/dL)	65	38	74 (1–1996)	27	227 (3–1534)	0.22
Fibrinogen (mg/dL)	383	253	300 (100–1320)	130	400 (100–1016)	**0.0003**
Leukocytes (cells/μL)	442	284	6850 (550–94 400)	158	5600 (800–31 980)	**0.001**
Neutrophils (cells/μL)	430	283	5180 (20–84 960)	147	4260 (40–28 142)	**0.009**
Band neutrophils (cells/μL)	225	134	0 (0–21 000)	91	1 (0–2000)	**0.01**
Band neutrophils (% of total)	224	133	0 (0–99.8)	91	0 (0–75.1)	0.03
Band neutrophils present	225	134	30.6% (41/134)	91	49.5% (45/91)	**0.01**
Toxic neutrophils present	330	224	31.7% (71/224)	106	55.6% (60/106)	**< 0.0001**
Lymphocytes (cells/μL)	432	284	1310 (48–8500)	148	1094 (200–8000)	**0.001**
Neutrophil to lymphocyte ratio	429	283	3.9 (0.1–46.0)	139	4.0 (0.1–23.6)	0.64
Monocytes (cells/μL)	324	276	200 (0–1770)	148	152 (0–1890)	**0.001**
Eosinophils (cells/μL)	424	277	0 (0–600)	147	0 (0–524)	0.79
Basophils (cells/μL)	414	269	0 (0–140)	145	0 (0–167)	0.66

(Continues)

TABLE 2 | (Continued)

Laboratory values	*N*	*n*	No infection % or median and range	*n*	Infection % or median and range	*p*
Platelets (cells/μL)	219	139	186 000 (15 600–463 000)	80	166 500 (12 000 – 426 000)	0.02
Duration of hospitalization (days)	447	288	5 (0–40)	159	5 (0–41)	0.86
Died/Euthanized	455	291	21.0% (61/291)	164	27.4% (45/164)	0.12

Note: Infection was defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus at a non-blood site on admission. Data were obtained from the medical records of 16 referral veterinary hospitals over 2 years. *N* is the total number of foals with data available for the study variable; *n* is the number of foals with data available that did not have evidence of infection or had an infection identified on admission. Variables and their associated *p* values that are significantly ($p < 0.01$) associated with infection are presented in bold font.

TABLE 3 | Multivariate logistic regression models for identifying associations of study variables with infection for 455 critically ill newborn foals < 3 days of age.

Variable	Coefficient	± SE	OR	95% CI for OR	*p*
Signalment, history, and physical examination model[a]					
Intercept	−1.01	0.15			< 0.0001
Foal age (hours)	0.022	0.005	1.02	1.01–1.03	< 0.0001
Laboratory analysis model[b]					
Intercept	−1.39	0.20			
Presence of toxic neutrophils	1.12	0.28	3.08	1.79–5.28	< 0.0001
All data model[c]					
Intercept	−1.67	0.23			< 0.0001
Foal age (hours)	0.020	0.007	1.02	1.01–1.03	< 0.0001
Presence of toxic neutrophils	0.88	0.29	2.41	1.37–4.26	< 0.0001

Note: Infection was defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus at a non-blood site on admission.
[a]Hosmer-Lemeshow goodness-of-fit $\chi^2 = 4.74$, df = 8, $p = 0.79$. AUC = 0.62.
[b]Hosmer-Lemeshow goodness-of-fit indices not able to be calculated. AUC = 0.64.
[c]Hosmer-Lemeshow goodness-of-fit $\chi^2 = 5.84$, df = 9, $p = 0.76$. AUC = 0.69.

best laboratory predictor of infection was the presence of toxic neutrophils. This finding was similar to that in a study of neonatal calves with diarrhea that identified toxic neutrophils as a predictor of sepsis [18]. Developing a standardized practical method for defining the presence of toxic neutrophils in foals would appear to be a useful clinical goal.

A positive blood culture provides valuable clinical information to formulate an optimal treatment plan for critically ill foals. Nevertheless, blood cultures are expensive to collect and analyze, with results not being available for 2 or more days. Our blood culture positivity rate of 27% was similar to that reported elsewhere over the last 6 years for foals admitted to referral hospitals, with positivity rates of 23% in foals < 7 days of age [19], 33% in foals < 14 days of age [20], and 41% in foals < 30 days of age [21], but lower than that in another study (64%) in foals < 3 days of age [21]. Most prior studies have considered positive blood culture as the reference ('gold standard') method for identifying a foal with systemic infection. False negative assignment in our study (negative blood culture in foals with infection) could have resulted from the collection of an inadequate blood volume for culture or inappropriate culture methodology [22, 23]. False positive assignment in our study (positive blood culture in foals without infection) could reflect contamination during collection [22]. For example, the overall contamination rate in critically ill calves recently was estimated to be 15% [23]. Bacteremia also could originate from placentitis [24] or be the result of nonspecific pinocytosis of pathogenic and non-pathogenic bacteria associated with the active transport of colostral immunoglobulins, as demonstrated in neonatal calves [25] and suspected to occur in some foals [26]. A novel finding of our study was that the probability of infection (predominantly reflecting bacteremia) increased in an approximately linear manner over the first 3 days of life. We speculate that this finding could reflect sustained translocation of bacteria from the small intestinal lumen into the blood stream and subsequent multiplication to a sufficient number of colony-forming units per millileter to be detected using standard blood culture methods.

The 1991 [5] and 2001 [27] sepsis consensus conferences developed and then re-evaluated the definition of sepsis in people as the presence of infection coupled with the patient fulfilling two or more of four SIRS criteria. As a result, SIRS became central to identifying sepsis in critically ill foals [8, 28, 29]. However, in 2016, the third international consensus definitions for sepsis and septic shock (Sepsis-3) redefined sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection [30]. A key change in that consensus meeting was that

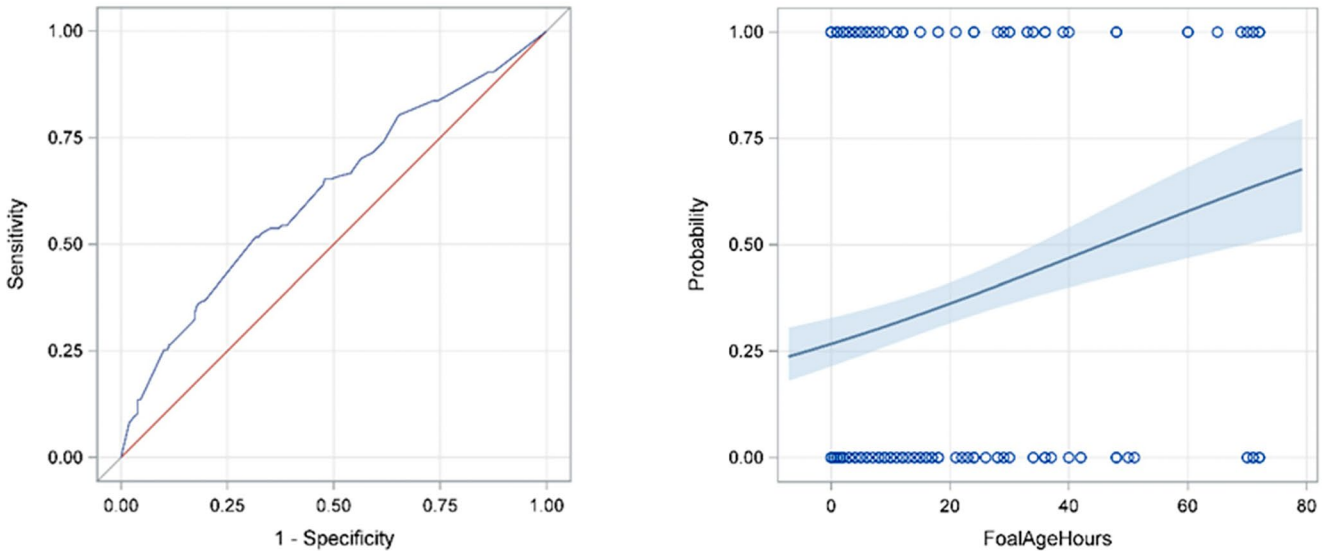

FIGURE 1 | Receiver operating characteristic (ROC) curve for predicting infection in 455 critically ill newborn foals. Infection was defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus at a non-blood site on admission. The left panel is the ROC curve for the logistic regression model obtained using history, signalment and physical examination data when available for > 50% of foals ($n = 406$; ROC = 0.62; red diagonal line indicates a test of no predictive value). At the optimal cut point (foal age > 55 h), Se = 0.52, Sp = 0.68, and accuracy = 0.62. The probability of infection increased almost linearly with age over the first 3 days (right panel; the blue shaded area represents the 95% confidence interval for prediction; open circles indicate the age of the foal on admission).

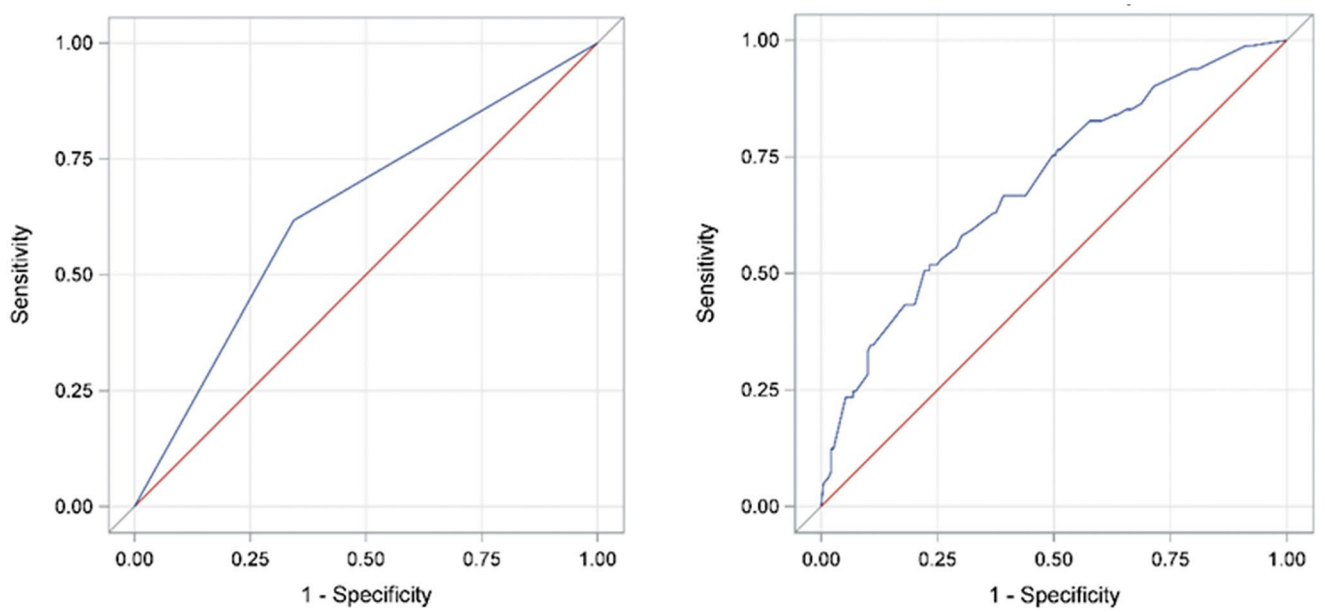

FIGURE 2 | Receiver operating characteristic (ROC) curves for predicting infection in 455 critically ill newborn foals. Infection was defined as the presence of bacteremia (positive blood culture) or clinical identification of an infected focus at a non-blood site on admission. The left panel is the ROC curve for the logistic regression model obtained using laboratory data when available for > 50% of foals ($n = 270$; ROC = 0.64; red diagonal line indicates a test of no predictive value). At the optimal cut point (toxic neutrophils present), Se = 0.62, Sp = 0.66, and accuracy = 0.64. The right panel is the ROC curve for the logistic regression model obtained using all the data when available for > 50% of foals ($n = 270$; ROC = 0.69). At the optimal cutpoint (toxic neutrophils present and foal age > 55 h), Se = 0.67, Sp = 0.61, and accuracy = 0.63.

the previous definition of sepsis had an excessive focus on inflammation and inadequate Sp and Se related to SIRS criteria. Hence, the participants in Sepsis-3 unanimously considered the use of SIRS criteria to be unhelpful and its use has been eliminated in critical care in human medicine. The participants stated that sepsis involves organ dysfunction, and the pathobiology was more complex than infection plus an accompanying inflammatory response alone [30]. Veterinarians historically have used the SIRS criteria as part of the definition of sepsis [6–10], but based on our results, we suggest that the SIRS criteria should not be used as part of the definition of sepsis in foals for the same reason as that noted in people [30] (i. e., low Se and Sp). Moreover, because we cannot rapidly predict the presence of infection in an individual foal with sufficient accuracy, it may be

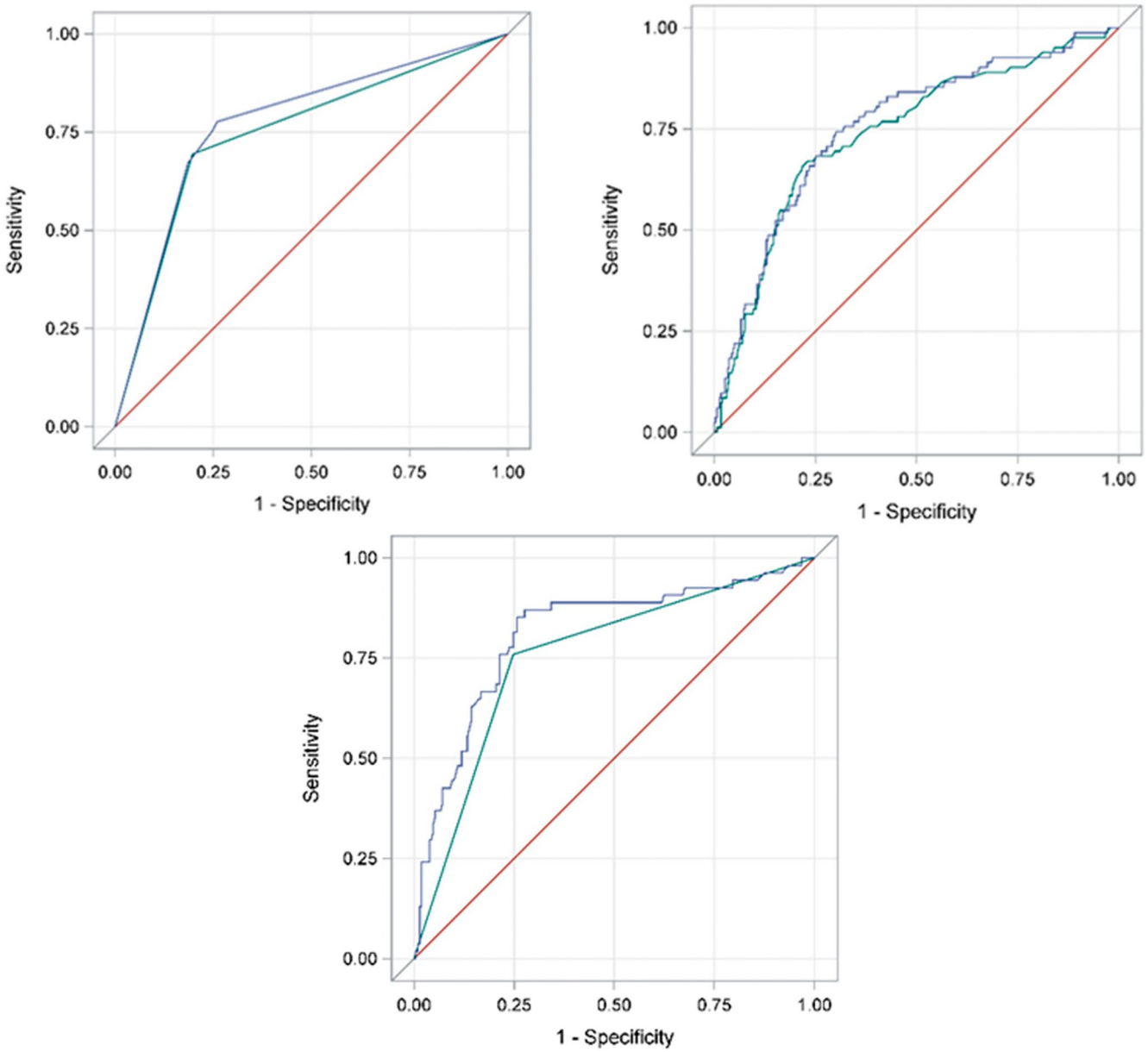

FIGURE 3 | Receiver operating characteristic (ROC) curves for predicting in-hospital mortality of critically ill newborn foals. The left panel is the ROC curve for the logistic regression model obtained using history, signalment and physical examination data when available for > 50% of foals ($n = 420$; ROC = 0.77; predictors are hypokinetic pulses and cold extremities). The right panel is the ROC curve for the logistic regression model obtained using laboratory data when available for > 50% of foals ($n = 384$; ROC = 0.76; predictors are increased blood L-lactate concentration and potassium concentration). The bottom panel is the ROC curve for the logistic regression model obtained using all data when available for > 50% of foals ($n = 264$; ROC = 0.81; predictors are hypokinetic pulses and increased total bilirubin concentration).

most appropriate to routinely implement parenteral antimicrobial treatment in critically ill newborn foals on welfare grounds. This recommendation is based on an approximate prevalence of bacteremia in critically ill foals of 30% and an in-hospital mortality rate of approximately 20%. This has been the recommended approach for decades in treating critically ill neonatal foals [3, 4] and neonatal calves with diarrhea, where the prevalence of bacteremia also approximates 30% [31]. This treatment approach needs to be balanced by an overarching concern for antimicrobial stewardship.

The overall survival rate (76%) was high in our study relative to earlier studies [4, 32], but similar to that in recently published studies (72%–83%) [9, 10, 19, 20], suggesting implementation of effective diagnostic and treatment protocols or earlier admission of sick foals. The presence of hypokinetic pulses was the strongest predictor of in-hospital mortality in our study; a weak or absent peripheral pulse previously was reported to be predictive of non-survival in foals < 7 days of age [33]. Hypokinetic pulses were suspected to reflect the presence of decreased systemic vascular tone, which is the hallmark of sepsis [15]. Hypokinetic pulses also can result from inadequate cardiac output because of decreased heart rate or the presence of cardiac arrhythmias, decreased venous return secondary to hypovolemia and dehydration, impaired myocardial contractility, incompetent heart valves, or a congenital cardiac defect. In contrast, a localized

TABLE 4 | Multivariate logistic regression models for identifying associations of study variables with in-hospital mortality in 1068 critically ill newborn foals.

Variable	Coefficient	±SE	OR	95% CI for OR	*p*
Signalment, history, and physical examination model[a]					
Intercept	−2.53	0.23			<0.0001
Hypokinetic pulses	1.19	0.44	3.28	(1.38–7.82)	0.01
Cold extremities	1.28	0.46	3.59	(1.47–8.80)	0.01
Laboratory analysis model[b]					
Intercept	−4.69	0.75			<0.0001
L-lactate concentration (mmol/L)	0.16	0.03	1.17	(1.10–1.24)	<0.0001
Potassium concentration (mmol/L)	0.60	0.17	1.83	(1.31–2.57)	0.01
All data model[c]					
Intercept	−3.55	0.46			<0.0001
Hypokinetic pulses	2.33	0.37	10.23	(4.92–21.26)	<0.0001
Total bilirubin concentration (mg/dL)	0.22	0.07	1.25	(1.10–1.42)	0.001

Note: Variables considered in each logistic regression model were $p < 0.05$ on univariate and categorical analysis, with data being available for > 50% of foals. Because of missing data, the final logistic regression models included 420 foals (signalment, history, and physical examination model), 384 foals (laboratory analysis model), and 264 foals (all data model).
[a]Hosmer-Lemeshow goodness-of-fit $\chi^2 = 0.44$, df = 1, $p = 0.51$. AUC = 0.77.
[b]Hosmer-Lemeshow goodness-of-fit $\chi^2 = 8.27$, df = 8, $p = 0.41$. AUC = 0.76.
[c]Hosmer-Lemeshow goodness-of-fit $\chi^2 = 5.59$, df = 8, $p = 0.69$. AUC = 0.81.

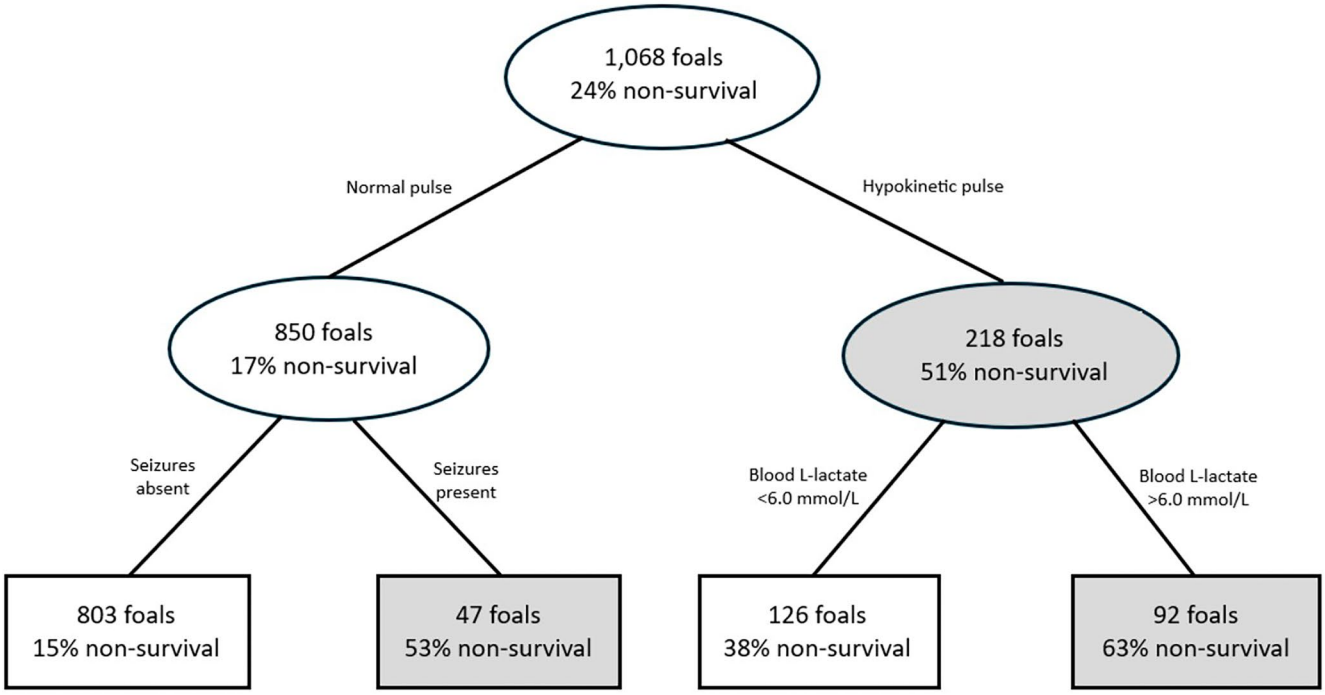

FIGURE 4 | Classification tree-derived decision tree for in-hospital mortality in 1068 critically ill newborn foals. Each node includes the total number of foals and the number of foals with in-hospital mortality, expressed as a percentage. Branches to the left in a split reflect a lower probability of mortality, whereas branches to the right (gray shading) reflect a higher probability of mortality. Rectangles represent subgroups that are not able to be further subdivided. The presence of hypokinetic pulses was the strongest predictor of in-hospital mortality, with increased mortality associated with hyperlactatemia (blood L-lactate concentration > 6.0 mmol/L). In contrast, in foals with normal arterial pulse pressures as assessed by palpation, the presence of seizures was also predictive of in-hospital mortality.

hypokinetic pulse indicates inadequate blood flow because of regional arterial pathology, such as vascular trauma causedby a fracture, or vascular occlusion caused by thrombosis. The clinical importance of systemic hypotension in septic humans has been emphasized [15] and documented in critically ill foals [34] and neonatal calves [35], and the presence of systemic hypotension most likely reflects the progression of sepsis to septic shock or an inadequate clinical response to IV fluid administration.

The presence of cold extremities was also a strong predictor of non-survival in our study and 2 earlier foal studies [33, 34] and most likely reflects inadequate cardiac output, and perhaps septic shock, in foals with core temperatures below the reference range. Fetlock temperature and core-peripheral temperature difference were linearly related to cardiac output in dehydrated calves when cardiac output was <65% of healthy euhydrated calves housed in a temperature-controlled environment [36]. Our findings therefore raise the question as to whether foal survival rates can be further improved with goal-directed treatment related to the optimal use of IV fluids and vasopressor agents [37, 38].

Hyperlactatemia is well-established as a biomarker for tissue hypoxia, disease severity, and mortality in human and veterinary medicine [39, 40]. Increased blood or plasma L-lactate concentrations are clinically useful indicators of non-survival in critically ill neonatal foals admitted to intensive care units (ICU) for treatment of prematurity, perinatal asphyxia, bacteremia, or enteritis [28, 41, 42]. Our classification tree analysis identified that blood L-lactate concentration >6.0 mmol/L was the most important laboratory finding associated with in-hospital mortality. Serial L-lactate measurements were of more prognostic value in septic children than a single measurement of L-lactate on admission [43]. Similar findings have been reported for critically ill foals [42, 44, 45] where delayed normalization or persistent L-lactatemia represents a more reliable indicator of non-survival than admission L-lactate concentration. It is therefore likely that serial measurements of blood or plasma L-lactate concentrations in critically ill newborn foals will provide more accurate prognostic information than a single measurement obtained before the initiation of treatment.

Hypoglycemia has been associated with sepsis and a low survival rate in neonatal foals and calves [46, 47]. This finding also was noted in our study in which non-surviving foals had lower blood glucose concentrations compared with foals that survived. Hypoglycemia can result from various causes in septic patients including increased utilization of peripheral glucose, depletion of glycogen reserves, decreased gluconeogenesis, or comparatively decreased nutrient supply [48–50]. Increased potassium and total bilirubin concentrations were identified as independent predictors of in-hospital mortality on logistic regression. Critically ill hyperkalemic humans with severe sepsis or septic shock had a higher ICU mortality rate and incidence of ventricular arrhythmias than patients with normal plasma potassium concentrations [51]. Hyperkalemia in neonatal calves [52], and presumably neonatal foals, can result from actual or relative hypovolemia, decreased glomerular filtration rate, and acid–base disturbances. Increased plasma potassium concentrations also can result from movement of intracellular potassium to the extracellular fluid compartment through ATP-sensitive potassium (K_{ATP}) channels, and excessive activation of K_{ATP} channels is recognized as a major cause of hypotension and vascular hyporesponsiveness to catecholamines in septic shock [53].

Increased serum total bilirubin concentration was an independent predictor of in-hospital mortality in foals in our study. Physiologic hyperbilirubinemia is common in healthy newborn and neonatal foals and is considered a physiologic phenomenon that is caused, in part, by post-natal removal of fetal erythrocytes [54]. Hyperbilirubinemia could have been associated with decreased feed intake, which was not evaluated as a predictor in our study, because fasting induces hyperbilirubinemia in adult horses [55]. However, we could not identify any studies that documented whether or not fasting-induced hyperbilirubinemia also occurs in foals. Hepatic dysfunction is common in septic humans [56] where it has been attributed to persistent microcirculatory failure and overactivation of the systemic response [57]. Hepatic injury is common in septic foals but was not predictive of in-hospital mortality in a previous study [58] and did not appear to contribute to mortality in foals in our study because serum aspartate aminotransferase and gamma glutamyltransferase activities were similar or lower, respectively, in non-surviving foals relative to survivors. Non-surviving foals in our study also had higher serum phosphate and creatinine concentrations, and increased serum creatine kinase activity than survivors on admission; similar findings have been reported previously [59]. These changes were likely the result of prerenal azotemia secondary to sepsis and septic shock, as well as prolonged recumbency in sick foals.

In our study, critically ill colt foals comprised 59% of total newborn admissions. Interestingly, a similar sex bias (59% to 61% colts) in admissions has been reported in at least 3 previous studies of critically ill foals [10, 19, 33]. The reason for this sex predilection is not fully known, but referral bias towards colt foals, sex differences in the immune system, or the impact of sex steroids may be contributing factors [21]. A notable sex dimorphism in terms of response to trauma, shock and sepsis has been well documented [60].

Our study had many strengths, including a large and diverse study population from 16 referral hospitals in 4 countries over 2 consecutive years, and the application of univariate and categorical comparison methods, logistic regression, and classification tree analysis. Limitations of the study include its retrospective nature, small group sizes for some study variables, use of in-hospital mortality as an outcome index when foals can be euthanized on welfare or financial grounds, and the subjective nature of identifying the presence of hypokinetic pulses and cold extremities. We do not consider our inability to calculate a sepsis score as proposed previously [13] to be a weakness. Almost all clinical scoring systems developed from two or more ordinal predictors are statistically invalid in that they incorrectly assume independence of predictors and equal weighting for all predictors. Statistical methods such as logistic regression and classification tree analysis can identify independent predictors and appropriately weight the predictors. Accordingly, we propose that logistic regression and classification tree analysis methods should replace clinical score systems when predicting clinical outcomes such as the likelihood of sepsis.

In conclusion, and contrary to our hypothesis, commonly applied SIRS indices such as abnormal rectal temperature, heart rate, respiratory rate, or leukocyte count were not predictive of infection in critically ill newborn foals in our study. In contrast, we identified many predictors of in-hospital mortality. Cardiovascular dysfunction, manifested by hypokinetic pulses, cold extremities, and hyperlactatemia, was strongly associated with in-hospital mortality of newborn foals.

Acknowledgments

The publication was a collaborative effort of attendees at the Dorothy Russell Havemeyer SIRS and Sepsis in Foals Working Group meeting at the University of Illinois Urbana Champaign in 2018. We thank Jonathan Palmer of University of Pennsylvania for his input.

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.

References

1. D. Wong, P. A. Wilkins, and G. Landolt, "Neonatal Infection," *Equine Neonatal Medicine* (2024): 1126–1177.

2. M. Miranda and S. Nadel, "Pediatric Sepsis: A Summary of Current Definitions and Management Recommendations," *Current Pediatrics Reports* 11, no. 2 (2023): 29–39.

3. C. L. Fielding and G. K. Magdesian, "Sepsis and Septic Shock in the Equine Neonate," *Veterinary Clinics of North America Equine Practice* 31 (2015): 483–496.

4. S. Taylor, "A Review of Equine Sepsis," *Equine Veterinary Education* 27 (2015): 99–109, https://doi.org/10.1111/eve.12290.

5. R. C. Bone, R. A. Balk, F. B. Cerra, et al., "Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis the ACCP/SCCM Consensus Conference Committee," *Chest* 101, no. 6 (1992): 1644–1655.

6. F. M. Trefz, I. Lorenz, A. Lorch, and P. D. Constable, "Clinical Signs, Profound Acidemia, Hypoglycemia, and Hypernatremia Are Predictive of Mortality in 1,400 Critically Ill Neonatal Calves With Diarrhea," *PLoS One* 12, no. 8 (2017): e0182938.

7. A. M. Spillane, J. L. Haraschak, S. E. Gephard, B. E. Nerderman, M. E. Fick, and J. M. Reinhart, "Evaluating the Clinical Utility of the Systemic Inflammatory Response Syndrome Criteria in Dogs and Cats Presenting to an Emergency Department," *Journal of Veterinary Emergency and Critical Care* 33, no. 3 (2023): 315–326.

8. D. M. Wong, R. E. Ruby, K. A. Dembek, et al., "Evaluation of Updated Sepsis Scoring Systems and Systemic Inflammatory Response Syndrome Criteria and Their Association With Sepsis in Equine Neonates," *Journal of Veterinary Internal Medicine* 32 (2018): 1185–1193.

9. M. Laurberg, C. Saegerman, S. Jacobsen, et al., "Use of Admission Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) Concentrations as a Marker of Sepsis and Outcome in Neonatal Foals," *PLoS One* 18, no. 5 (2023): e0285819.

10. E. Hoeberg, A. Sånge, C. Saegerman, et al., "Serum Amyloid A as a Marker to Detect Sepsis and Predict Outcome in Hospitalized Neonatal Foals," *Journal of Veterinary Internal Medicine* 36, no. 6 (2022): 2245–2253.

11. P. D. Constable, "A Simplified Strong Ion Model for Acid-Base Equilibria: Application to Horse Plasma," *Journal of Applied Physiology* 83, no. 1 (1997): 297–311.

12. P. D. Constable and K. W. Hinchcliff, "Comparison of Anion Gap and Strong Ion Gap as Predictors of Unmeasured Strong Ion Concentration in Plasma and Serum From Horses," *American Journal of Veterinary Research* 59, no. 7 (1998): 881–887.

13. B. D. Brewer and A. M. Koterba, "Development of a Scoring System for the Early Diagnosis of Equine Neonatal Sepsis," *Equine Veterinary Journal* 20, no. 1 (1988): 18–22.

14. G. Piccione, A. Assenza, A. Costa, et al., "Monitoring of Some Physiological Parameters During the First 30 Days of a Foal's Life," *Medycyna Weterynaryjna* 61 (2005): 1142–1144.

15. B. Goldstein, B. Giroir, and A. Randolph, "International Pediatric Sepsis Consensus Conference: Definitions for Sepsis and Organ Dysfunction in Pediatrics. International Consensus Conference on Pediatric Sepsis," *Pediatric Critical Care Medicine* 6 (2005): 2–8.

16. J. F. Hair, R. E. Anderson, R. L. Tatham, et al., *Multivariate Data Analysis*, 5th ed. (Prentice-Hall, Inc., 1998), 141–238.

17. J. A. Swets, "Measuring the Accuracy of Diagnostic Systems," *Science* 240 (1988): 1285–1293.

18. J. Lofstedt, I. R. Dohoo, and G. Duizer, "Model to Predict Septicemia in Diarrheic Calves," *Journal of Veterinary Internal Medicine* 13, no. 2 (1999): 81–88.

19. R. Scalco, G. N. de Oliveira, B. da Rosa Curcio, et al., "Red Blood Cell Distribution Width to Platelet Ratio in Neonatal Foals With Sepsis," *Journal of Veterinary Internal Medicine* 37, no. 4 (2023): 1552–1560.

20. M. Furr and H. McKenzie, III, "Factors Associated With the Risk of Positive Blood Culture in Neonatal Foals Presented to a Referral Center (2000-2014)," *Journal of Veterinary Internal Medicine* 34, no. 6 (2020): 2738–2750.

21. J. M. Swink, L. M. Rings, H. A. Snyder, et al., "Dynamics of Androgens in Healthy and Hospitalized Newborn Foals," *Journal of Veterinary Internal Medicine* 35, no. 1 (2021): 538–549.

22. S. Giancola and K. A. Hart, "Equine Blood Cultures: Can We Do Better?," *Equine Veterinary Journal* 55, no. 4 (2023): 584–592.

23. M. L. Pas, F. Boyen, D. Castelain, et al., "Bayesian Evaluation of Sensitivity and Specificity of Blood Culture Media and Hypoglycemia in Sepsis-Suspected Calves," *Journal of Veterinary Internal Medicine* 38 (2024): 1906–1916.

24. L. D. Borba, C. E. Nogueira, F. R. Bruhn, et al., "Peripheral Blood Markers of Sepsis in Foals Born From Mares With Experimentally Induced Ascending Placentitis," *Veterinary Record* 187, no. 1 (2020): 29.

25. L. D. Corley, T. E. Staley, L. J. Bush, and E. W. Jones, "Influence of Colostrum on Transepithelial Movement of *Escherichia coli* 055," *Journal of Dairy Science* 60 (1977): 1416–1421.

26. E. S. Hackett, D. P. Lunn, R. A. Ferris, D. W. Horohov, M. R. Lappin, and P. M. McCue, "Detection of Bacteraemia and Host Response in Healthy Neonatal Foals," *Equine Veterinary Journal* 47 (2015): 405–409.

27. M. M. Levy, M. P. Fink, J. C. Marshall, et al., "2001 Sccm/Esicm/Accp/Ats/Sis International Sepsis Definitions Conference," *Critical Care Medicine* 31 (2003): 1250–1256.

28. K. T. Corley, L. L. Donaldson, and M. O. Furr, "Arterial Lactate Concentration, Hospital Survival, Sepsis and SIRS in Critically Ill Neonatal Foals," *Equine Veterinary Journal* 37, no. 1 (2005): 53–59.

29. F. Bonelli, V. Meucci, T. J. Divers, et al., "Plasma Procalcitonin Concentration in Healthy Horses and Horses Affected by Systemic Inflammatory Response Syndrome," *Journal of Veterinary Internal Medicine* 29 (2015): 1689–1691.

30. M. Singer, C. S. Deutschman, C. W. Seymour, et al., "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)," *Journal of the American Medical Association* 315 (2016): 801–810.

31. P. D. Constable, "Antimicrobial Use in the Treatment of Calf Diarrhea," *Journal of Veterinary Internal Medicine* 18, no. 1 (2004): 8–17.

32. S. Giguère, E. J. Weber, and L. C. Sanchez, "Factors Associated With Outcome and Gradual Improvement in Survival Over Time in 1065 Equine Neonates Admitted to an Intensive Care Unit," *Equine Veterinary Journal* 49 (2017): 45–50.

33. S. D. Hurcombe, R. E. Toribio, N. M. Slovis, et al., "Calcium Regulating Hormones and Serum Calcium and Magnesium Concentrations in Septic and Critically Ill Foals and Their Association With Survival," *Journal of Veterinary Internal Medicine* 23, no. 2 (2009): 335–343.

34. K. A. Dembek, S. D. Hurcombe, A. J. Stewart, et al., "Association of Aldosterone and Arginine Vasopressin Concentrations and Clinical Markers of Hypoperfusion in Neonatal Foals," *Equine Veterinary Journal* 48 (2016): 176–181.

35. A. Naseri, I. Sen, K. Turgut, H. Guzelbektes, and P. D. Constable, "Echocardiographic Assessment of Left Ventricular Systolic Function in Neonatal Calves With Naturally Occurring Sepsis or Septic Shock due to Diarrhea," *Research in Veterinary Science* 126 (2019): 103–112.

36. P. D. Constable, P. G. Walker, D. E. Morin, J. H. Foreman, and J. C. Thurmon, "Use of Peripheral Temperature and Core-Peripheral Temperature Difference to Predict Cardiac Output in Dehydrated Calves Housed in a Thermoneutral Environment," *American Journal of Veterinary Research* 59, no. 7 (1998): 874–880.

37. Y. S. Nout, K. T. Corley, L. L. Donaldson, and M. O. Furr, "Indirect Oscillometric and Direct Blood Pressure Measurements in Anesthetized and Conscious Neonatal Foals," *Journal of Veterinary Emergency and Critical Care* 12, no. 2 (2002): 75–80.

38. A. Valverde, S. Giguère, L. C. Sanchez, A. Shih, and C. Ryan, "Effects of Dobutamine, Norepinephrine, and Vasopressin on Cardiovascular Function in Anesthetized Neonatal Foals With Induced Hypotension," *American Journal of Veterinary Research* 67, no. 10 (2006): 1730–1737.

39. J. A. Kraut and N. E. Madias, "Lactic Acidosis: Current Treatments and Future Directions," *American Journal of Kidney Diseases* 68, no. 3 (2016): 473–482, https://doi.org/10.1053/j.ajkd.2016.04.020.

40. D. S. Pang and S. Boysen, "Lactate in Veterinary Critical Care: Pathophysiology and Management," *Journal of the American Animal Hospital Association* 43, no. 5 (2007): 270–279, https://doi.org/10.5326/0430270.

41. C. Castagnetti, A. Pirrone, J. Mariella, and G. Mari, "Venous Blood Lactate Evaluation in Equine Neonatal Intensive Care," *Theriogenology* 73, no. 3 (2010): 343–357.

42. A. Borchers, P. Wilkins, P. Marsh, et al., "Sequential L-Lactate Concentration in Hospitalised Equine Neonates: A Prospective Multicentre Study," *Equine Veterinary Journal* 45, no. S45 (2013): 2–7.

43. T. D. Duke, W. Butt, and M. South, "Predictors of Mortality and Multiple Organ Failure in Children With Sepsis," *Intensive Care Medicine* 23, no. 6 (1997): 684–692.

44. K. Wotman, P. A. Wilkins, J. E. Palmer, and R. C. Boston, "Association of Blood Lactate Concentration and Outcome in Foals," *Journal of Veterinary Internal Medicine* 23, no. 3 (2009): 598–605, https://doi.org/10.1111/j.1939-1676.2009.0277.x.

45. P. A. Wilkins, B. J. Sheahan, K. A. Vander Werf, et al., "Preliminary Investigation of the Area Under the L-Lactate Concentration-Time Curve (LACAREA) in Critically Ill Equine Neonates," *Journal of Veterinary Internal Medicine* 29 (2015): 659–662.

46. A. R. Hollis, M. O. Furr, K. G. Magdesian, et al., "Blood Glucose Concentrations in Critically Ill Neonatal Foals," *Journal of Veterinary Internal Medicine* 22, no. 5 (2008): 1223–1227, https://doi.org/10.1111/j.1939-1676.2008.0174.x.

47. F. M. Trefz, M. Feist, and I. Lorenz, "Hypoglycaemia in Hospitalised Neonatal Calves: Prevalence, Associated Conditions and Impact on Prognosis," *Veterinary Journal* 217 (2016): 103–108, https://doi.org/10.1016/j.tvjl.2016.10.001 PMID: 27810199.

48. K. Dendoncker and C. Libert, "Glucocorticoid Resistance as a Major Drive in Sepsis Pathology," *Cytokine and Growth Factor Reviews* 35 (2017): 85–96.

49. S. Weis, A. R. Carlos, M. R. Moita, et al., "Metabolic Adaptation Establishes Disease Tolerance to Sepsis," *Cell* 169 (2017): 1263–1275.

50. W. Wasyluk and A. Zwolak, "Metabolic Alterations in Sepsis," *Journal of Clinical Medicine* 10 (2021): 2412.

51. S. Tongyoo, T. Viarasilpa, and C. Permpikul, "Serum Potassium Levels and Outcomes in Critically Ill Patients in the Medical Intensive Care Unit," *Journal of International Medical Research* 46, no. 3 (2018): 1254–1262.

52. F. M. Trefz, P. D. Constable, C. Sauter-Louis, A. Lorch, G. Knubben-Schweizer, and I. Lorenz, "Hyperkalemia in Neonatal Diarrheic Calves Depends on the Degree of Dehydration and the Cause of the Metabolic Acidosis but Does Not Require the Presence of Acidemia," *Journal of Dairy Science* 96, no. 11 (2013): 7234–7244.

53. J. F. Buckley, M. Singer, and L. H. Clapp, "Role of KATP Channels in Sepsis," *Cardiovascular Research* 72, no. 2 (2006): 220–230.

54. M. Hoffman, S. H. Cheong, and T. Stokol, "Transient Increases in Glutamate Dehydrogenase (GLDH) Activity Occur in Neonatal Foals," *Veterinary Clinical Pathology* 52 (2023): 261–270.

55. L. R. Engelking, M. S. Anwer, and J. Lofstedt, "Hepatobiliary Transport of Indocyanine Green and Sulfobromophthalein in Fed and Fasted Horses," *American Journal of Veterinary Research* 46 (1985): 2278–2284.

56. J. J. Patel, A. Taneja, D. Niccum, G. Kumar, E. Jacobs, and R. Nanchal, "The Association of Serum Bilirubin Levels on the Outcomes of Severe Sepsis," *Journal of Intensive Care Medicine* 30, no. 1 (2015): 23–29.

57. D. Wang, Y. Yin, and Y. Yao, "Advances in Sepsis-Associated Liver Dysfunction," *Burn Trauma* 2 (2014): 97–105.

58. E. F. Haggett, K. G. Magdesian, and P. H. Kass, "Clinical Implications of High Liver Enzyme Activities in Hospitalized Neonatal Foals," *Journal of the American Veterinary Medical Association* 239 (2011): 661–667.

59. L. C. Sanchez, S. Giguère, and G. D. Lester, "Factors Associated With Survival of Neonatal Foals With Bacteremia and Racing Performance of Surviving Thoroughbreds: 423 Cases (1982-2007)," *Journal of the American Veterinary Medical Association* 233, no. 9 (2008): 1446–1452.

60. F. Bosch, M. K. Angele, and I. Chaudry, "Gender Differences in Trauma, Shock and Sepsis," *Military Medical Research* 5 (2018), https://doi.org/10.1186/s40779-018-0182-5.

Supporting Information

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