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Sepsis and survival in critically ill calves: Risk factors and antimicrobial use

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Abstract

Background: Sepsis is a life-threatening disease for which critically important antimicrobials (CIA) frequently are used. Diagnostic and therapeutic guidelines for sepsis and critically ill calves are largely lacking.

Objectives: Identify factors associated with mortality in critically ill calves and describe bacteria obtained from blood cultures of critically ill calves with sepsis and their antimicrobial resistance.

Animals: Two-hundred thirty critically ill calves, mainly Belgian Blue beef cattle.

Methods: Retrospective cohort study. Logistic regression, survival analysis, and decision tree analysis were used to determine factors associated with mortality.

Results: Of the critically ill calves, 34.3% had sepsis and 61.3% died. The final survival model indicated that calves with sepsis (hazard risk [HR]: 1.6; 95% confidence interval [CI]: 1.0-2.5; $P = .05$), abnormal behavior (HR: 2.3; 95% CI: 1.3-4.0; $P = .005$), and hypothermia (HR: 0.82; 95% CI: 0.72-0.95; $P = .01$) had a significantly higher mortality risk. In a second survival model, hypothermia (HR: 0.87; 95% CI: 0.78-0.96; $P = .004$) and hypoglycemia (HR: 2.2; 95% CI: 1.5-3.3; $P < .001$) were risk factors for mortality. Decision tree analysis emphasized the importance of behavior, hypochloremia, hypoglycemia, hyperkalemia, and lung ultrasonography for mortality risk.

Escherichia coli (30.6%) was most frequently isolated from blood cultures, of which 90.9% were multidrug resistant. Inappropriate use of antimicrobials was frequent for penicillin, amoxicillin, and sulfamethoxazole/trimethoprim, but less for CIA.

Conclusions and Clinical Importance: Many critically ill calves have sepsis, which increases mortality risk. Bacteria involved are often resistant to first-intention antimicrobials but less resistant to CIA. The other identified risk factors for mortality can support therapeutic decision-making.

KEYWORDS
antimicrobial resistance, cattle, critically important antimicrobials, mortality

Abbreviations: AST, antimicrobial susceptibility testing; AUC, area under the curve; BB, Belgian Blue; CI, confidence interval; CIA, critically important antimicrobials; CART, classification and regression tree; HF, Holstein Friesian; MDR, multidrug resistance; NRDS, neonatal respiratory distress syndrome; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; R, range; ROC, receiver operating characteristics; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; SXT, sulfamethoxazole/trimethoprim.

1 | INTRODUCTION

Sepsis is the life-threatening dysregulated response of the body to infectious pathogens or their toxins present in the bloodstream, and a leading cause of mortality in animals and humans.[1-5] Sepsis is poorly explored in farm animals, but older studies estimate that up to 30% of calves with neonatal diarrhea or illness are septic.[6,7] Guidelines from human medicine emphasize the importance of rapid IV broad-spectrum bactericidal antimicrobial treatment, followed by timely antimicrobial de-escalation to guarantee future effectiveness and counter resistance selection against these products.[8-10] Antimicrobial use can be considered appropriate or inappropriate. The latter being defined as use of an antimicrobial agent to which the causal pathogen is resistant.[11] In humans, inappropriate treatment and delayed initiation of treatment are associated with decreased survival.[11-13] To treat sepsis, critically important antimicrobials (CIA), such as cephalosporins and fluoroquinolones, frequently are preferred in veterinary practice. However, public concern about antimicrobial use in farm animals warrants decreased use of these CIA in light of the One Health approach.[14,15] Also, different national guidelines for antimicrobial treatment in cattle recommend the use of first intention treatment with, for example, potentiated sulphonamides to safeguard fluoroquinolones and cephalosporins as last resort agents.[16,17] In Belgium, the Netherlands and Germany, fluoroquinolones and cephalosporins can only be used in farm animals under strict legal conditions, mostly including the requirement of microbiological testing.[18-20] Blood culture is preferred for the confirmation of sepsis and in humans its use significantly increases the chance of survival.[6,10,21] Information on bacteria involved in sepsis in calves and their antimicrobial resistance profile is scarce. Most studies are outdated and none are available for the European Union, where legislation on CIA is most strict.[7,22-24] Regional resistance data could help avoid inappropriate or unnecessary antimicrobial use, unnecessary costs, and adverse effects of these medications.[6] Calf mortality is an important welfare and economic problem, but it is unclear to what extent sepsis increases mortality risk in calves.[25] Moreover, risk factors for mortality in critically ill calves rarely are documented.[26-28] Information on the prognosis in critically ill calves can contribute to more rational decision-making to initiate treatment, taking economic, animal welfare, and antimicrobial resistance aspects into account. Therefore, our first objective was to determine factors associated with mortality in critically ill calves. Our second objective was to describe the bacteria involved in critically ill calves with sepsis and their antimicrobial resistance profiles.

2 | MATERIALS AND METHODS

2.1 | Study design and inclusion criteria

A retrospective cohort study was conducted on the medical records of critically ill calves presented between February 2017 and December 2020 in the university clinic, because from 2017 routine blood culturing from critically ill calves to confirm sepsis was performed. Inclusion criteria for the calves were an age <130 days and critical illness.

2.2 | Definitions

Critical illness was defined as severe respiratory, cardiovascular, or neurological abnormalities (apathy, decubitus, or neurological signs), alone or in combination.[29] Sepsis was defined as a positive blood culture in combination with critical illness. Neonatal respiratory distress syndrome (NRDS), to which Belgian Blue cattle are predisposed, was defined as respiratory distress a few hours after birth, caused by surfactant deficiency.[30,31] Diagnosis was based on historical information, clinical examination, blood tests, and ultrasound examination of the animal. Comorbidity was defined as the presence of multiple health conditions in the animal. No index disease was taken into account (i.e., no distinction was made for multimorbidity).[32]

Multidrug resistance (MDR) was defined as a pathogen resistant to at least 1 agent belonging to 3 different antimicrobial classes; intrinsic resistance was not included.[33] Inappropriate antimicrobial treatment was defined as the use of an antimicrobial to which the pathogen obtained from the blood culture was resistant (i.e., low chance of clinical efficacy due either to acquired or intrinsic resistance).[11] We further differentiated between theoretical and observed appropriateness. Theoretical appropriateness was calculated for all antimicrobials, regardless of whether this antimicrobial was used in the respective animal. In contrast, observed appropriateness was limited to cases where the specific antimicrobial was given to the animal, and thus indicates whether the administered antimicrobial was effective in the animal. Similar to a previous study,[33] penicillins and cephalosporins were included as 2 classes (not combined as β-lactam agents). Sulfamethoxazole/trimethoprim (SXT) was considered 1 class, given that during testing with disk diffusion mainly combination disks were used, making separate interpretation impossible. Included antimicrobials in the analysis were: penicillins, cephalosporins, macrolides, lincosamides, aminoglycosides, phenicols, quinolones, tetracyclines, and SXT.

2.3 | Clinical and blood examination

Clinical examination was performed on the day of blood culture sampling.[34] Three demographic factors (breed, age, sex), 15 clinical factors (temperature [°C], heart rate [beats/min], respiratory rate [breaths/min], mucosa color [pink, pink pale, congested], capillary filling time [seconds], skin pinch test [seconds], enophthalmos [mm], abnormal behavior, congestion of scleral vessels, and presence of morbidities such as arthritis, phlebitis, omphalitis, diarrhea, enteritis, and pneumonia) were evaluated during clinical examination. For phlebitis, the jugular vein was evaluated for swelling or inflammation. Dehydration was determined on the duration of the prolonged turgor time and degree of enophthalmos (mm). Pneumonia and enteritis were evaluated by ultrasound examination, using a linear 7.5 MHz probe (Easote

TABLE 1 Mortality and sepsis risk in 230 critically ill calves according to diagnosis and comorbidity

Diagnosis	Percentage (N)	Mortality % (N)	Sepsis % (N)
Enteritis/diarrhea total	51.7 (119)	54.6 (65)	29.4 (35)
Only enteritis/diarrhea	13.0 (30)	53.3 (16)	40.0 (12)
Comorbidity with pneumonia	13.9 (32)	59.4 (19)	31.3 (10)
Comorbidity with pneumonia + omphalitis	4.3 (10)	70.0 (7)	20.0 (2)
Comorbidity with pneumonia + NRDS	1.3 (3)	66.6 (2)	33.3 (1)
Comorbidity with pneumonia + others *Including phlebitis* (2), *hepatitis* (3), *arthritis + nephritis*, *pleuritis*, *abomasitis*	3.5 (8)	37.5 (3)	12.5 (1)
Comorbidity with NRDS	3.9 (9)	33.3 (3)	22.2 (2)
Comorbidity with NRDS + omphalitis	2.2 (5)	60.0 (3)	0 (0)
Comorbidity with NRDS + hepatitis	0.4 (1)	100.0 (1)	0 (0)
Comorbidity with omphalitis	2.2 (5)	.2 (1)	40.0 (2)
Comorbidity with omphalitis + others *Including BNP*, *hepatitis*, *splenitis*, *phlebitis*	1.7 (4)	75.0 (3)	25.0 (1)
Comorbidity with others *Including abomasitis*, *BNP*, *pleuritis*, *cerebrocortical necrosis*, *abomasitis/ruminitis*, *hepatitis*, *peritonitis*, *nervous signs*, *salt intoxication*	5.2 (12)	50.0 (6)	33.3 (4)
Pneumonia total	45.7 (105)	68.6 (72)	39.0 (41)
Only pneumonia	10.0 (23)	73.9 (17)	43.5 (10)
Comorbidity with omphalitis	1.7 (4)	100.0 (4)	50.0 (2)
Comorbidity with omphalitis + NRDS	0.9 (2)	50.0 (1)	50.0 (1)
Comorbidity with omphalitis + arthritis	0.9 (2)	100.0 (2)	100.0 (2)
Comorbidity with omphalitis + others *Including peritonitis*, *cystitis + meningitis*, *paralysis hindquarters*	1.3 (3)	100.0 (3)	33.3 (1)
Comorbidity with NRDS	3.9 (9)	66.7 (6)	55.6 (5)
Comorbidity with phlebitis	1.3 (3)	100.0 (3)	66.6 (2)
Comorbidity with others *Including abomasitis*, *arthritis*, *lead toxicity*, *peritonitis*, *pleuritis*, *pleuritis + osteomyelitis*	2.6 (6)	66.7 (4)	66.7 (4)
NRDS total	17.8 (41)	53.7 (22)	22 (9)
Only NRDS	4.3 (10)	60.0 (6)	0 (0)
Comorbidity with omphalitis	0.9 (2)	50.0 (1)	0 (0)
Comorbidity with atresia ani	0.4 (1)	0 (0)	0 (0)
Omphalitis total	17.8 (41)	61.0 (25)	22.0 (9)
Only omphalitis	1.7 (4)	50.0 (2)	0 (0)
Comorbidity with arthritis	0.4 (1)	0 (0)	0 (0)
Comorbidity with nervous signs	0.4 (1)	50.0 (1)	0 (0)
Hemorrhage	1.3 (3)	66.7 (2)	0 (0)
Phlebitis	1.3 (3)	33.3 (1)	100.0 (3)
Arthritis	1.3 (3)	100.0 (3)	100.0 (3)
Others *Including abomasitis*, *Clostridium perfringens*, *hepatitis*, *renal failure*, *pleuritis*, *spinal fracture*, *nervous signs*, *otitis media*, *peritonitis* (*ulcer*), *peritonitis* (*atresia ani*), *abomasitis + phlebitis*, *hepatitis + abomasitis + ruminitis*	5.2 (12)	75.0 (9)	41.7 (5)
Unknown	8.3 (19)	63.2 (12)	31.6 (6)
Total	230	61.3 (141)	34.3 (79)

Abbreviations: N, number of calves; NRDS, neonatal respiratory distress syndrome.

TABLE 2 Results of univariable analysis of clinical factors associated with mortality in 230 critically ill calves

Variable	Categories	Observed mortality	*P*-value	OR	95% CI
Positive blood culture (sepsis)	Blood culture negative (ref)	53.6% (81/151)			
	Blood culture positive	76.9% (60/78)	**<.001**	2.9	1.6-5.3
Breed	Belgian Blue (ref)	64.7% (121/187)	.09		
	Holstein Friesian	40.0% (8/20)	**.04**	0.36	0.14-0.93
	Others	55.0% (11/20)	.39	0.67	0.26-1.7
Age	<5.5 d (ref)	57.1% (60/105)			
	≥5.5 d	65.3% (81/124)	.21	1.4	0.83-2.4
Gender	Female (ref)	56.6% (60/106)			
	Male	68.5% (76/111)	.07	1.7	0.96-2.9
Temperature (continue)			**<.001**	0.62	0.48-0.79
Temperature (categorical)	38°C-39.9°C (ref)	58.6% (78/133)	**<.001**		
	<38°C	85.4% (35/41)	**.003**	4.1	1.6-10.4
	≥40°C	40.5% (15/37)	.05	0.48	0.23-1.0
Heart rate	<126 bpm (ref)	55.6% (60/108)			
	≥126 bpm	65.9% (54/82)	.15	1.5	0.85-2.8
Respiratory rate (continue)			.76	1.0	0.99-1.0
Respiratory rate (categoric)	30-45 bpm (ref)	51.1% (23/45)	.33		
	<30 bpm	65.7% (23/35)	.19	1.8	0.74-4.6
	>45 bpm	62.8% (70/112)	.19	1.6	0.79-3.2
Mucosae	Pink (ref)	53.5% (46/86)	**.003**		
	(Pink) pale	85.4% (35/41)	**<.001**	5.1	1.9-13.3
	Congested	52.3% (23/44)	.9	0.95	0.46-2.0
Capillary refill time	Normal (ref)	58.1% (43/74)			
	Prolonged (>2 sec)	67.2% (43/64)	.27	1.5	0.74-3.0
Turgor	Normal (ref)	47.8% (33/69)			
	Prolonged (>2 sec)	70.5% (67/95)	**.004**	2.6	1.4-5.0
Enophthalmos	Absent (ref)	53.3% (8/15)			
	Present	60.9% (14/23)	.65	1.4	0.37-5.1
Arthritis	Absent (ref)	60.0% (18/30)			
	Present	87.5% (7/8)	.17	4.7	0.51-42.9
Phlebitis	Absent (ref)	60.0% (18/30)			
	Present	60.0% (6/10)	1.0	1.0	0.23-4.3
Fecal consistency	Normal or pasty feces	56.9% (33/58)			
	Watery feces (incl. blood. fibrine....)	56.6% (30/53)	.98	0.99	0.47-2.1
Omphalitis	Absent (ref)	60.3% (35/58)			
	Present	63.4% (26/41)	.76	1.1	0.50-2.6
Scleral injections	Absent (ref)	52.2% (12/23)			
	Present	72.2% (13/18)	.20	2.4	0.64-8.9
Behavior	Normal and slightly lethargic (ref)	36.7% (18/49)			
	Abnormal. Cannot get up without help	77.8% (63/81)	**<.001**	6.0	2.8-13.2
Enteritis	Present (ref)	51.6% (32/62)			
	Absent	61.0% (47/77)	.27	1.5	0.75-2.9
Pneumonia	No lesions and consolidation <1 cm (ref)	54.9% (67/122)			
	Consolidation ≥1 cm	70.0% (49/70)	**.04**	1.9	1.0-3.6

Abbreviations: 95% CI, 95% confidence interval; bpm, beats or breaths per minute; d, day(s); OR, odds ratio; ref, reference.

TABLE 3 Results of univariable analysis of laboratory factors in venous blood associated with mortality in 230 critically ill calves

Variable	Categories	Observed mortality	*P*-value	OR	95% CI
pH	>7.18 (ref)	55.0% (72/131)			
	≤7.18	78.7% (37/47)	**.005**	3.0	1.4-6.6
pCO_2	<45.35 mm Hg (ref)	49.4% (38/77)			
	≥45.35 mm Hg	69.7% (69/99)	**.007**	2.4	1.3-4.4
pO_2 (mmHg)			.17	0.98	0.95-1.0
HCO_3	91.6-171.4 mg/dL (15.1-28.0 mmol/L) (ref)	55.1% (43/78)	.25		
	≤91.5 mg/dL (15.0 mmol/L)	71.1% (27/38)	.1	2.0	0.87-4.6
	≥171.5 mg/dL (28.1 mmol/L)	57.5% (23/40)	.81	1.1	0.51-2.4
Packed cell volume	<47.5% (ref)	57.0% (81/142)			
	≥47.5%	85.7% (18/21)	**.02**	4.5	1.3-16.0
Base excess	≥−5 mEq/L (ref)	51.5% (50/97)			
	<−5 mEq/L	69.0% (58/84)	**.02**	2.1	1.1-3.9
Hypoglycemia	>55 mg/dL (ref)	53.4% (71/133)	**<.001**	5.5	2.2-14.0
	≤55 mg/dL	86.4% (38/44)			
Sodium	<147.55 mEq/L (ref)	57.0% (81/142)			
	≥147.55 mEq/L	70.6% (24/34)	.15	1.8	0.81-4.1
Potassium	<6.08 mEq/L (ref)	57.7% (94/163)			
	≥6.08 mEq/L	90.0% (18/20)	**.01**	6.6	1.5-29.4
Chloride (mEq/L)			.16	0.97	0.93-1.0
Calcium	≥4.69 mg/dL (1.17 mmol/L) (ref)	55.9% (66/118)			
	<4.69 mg/dL (1.17 mmol/L)	73.2% (41/56)	**.03**	2.2	1.1-4.3
Lactate	<96.22 mg/L (10.68 mmol/L)	48.8% (20/41)			
	≥96.22 mg/L (10.68 mmol/L)	92.3% (12/13)	**.02**	12.6	1.5-106.0
Urea	<26.13 mg/dL (4.35 mmol/L)	52.2% (12/23)			
	≥26.13 mg/dL (4.35 mmol/L)	73.2% (30/41)	.09	2.5	0.86-7.3
Creatinine	<2.22 mg/dL (196 µmol/L)	50.0% (18/36)			
	≥2.22 mg/dL (196 µmol/L)	83.3% (25/30)	**.007**	5.0	1.6-16.0
Leukocytes	3.40-11.30 × 10^9/L	64.3% (9/14)	.27		
	≤3.39 × 10^9/L	85.7% (6/7)	.32	3.3	0.31-36.1
	≥11.31 × 10^9/L	44.4% (4/9)	.35	0.44	0.08-2.5
Neutrophils	2.1-12.3 × 10^9/L	61.5% (8/13)	.7		
	≤2.1 × 10^9/L	71.4% (5/7)	.66	1.6	0.22-11.4
	≥12.3 × 10^9/L	50.0% (4/8)	.61	0.63	0.11-3.7
Lymphocytes	1.2-10.6 × 10^9/L	55.6% (10/18)			
	<1.2 × 10^9/L	75.0% (9/12)	.29	2.4	0.48-11.9
	>10.6 × 10^9/L	/			
Thrombocytes	<359.5 K/µL	58.8% (10/17)			
	≥359.5 K/µL	75.0% (9/12)	.37	2.1	0.41-10.7
Total protein	≥5.5 g/dL	47.1% (16/34)			
	<5.5 g/dL	78.3% (18/23)	**.02**	4.1	1.2-13.4

Abbreviations: 95% CI, 95% confidence interval; bpm, beats or breaths per minute; d, day(s); OR, odds ratio; ref, reference.

MyLab30 Gold unit, the Netherlands or Sonosite M-Turbo, Fujifilm). For lung ultrasonography, both sides of the thorax were scanned, focusing on the presence of comet tail artifacts (few, multiple, diffuse) on the pleura as well as size of consolidations and presence of free fluid in the thorax. Umbilical infections were evaluated macroscopically and by ultrasound examination as previously described, distinguishing among local umbilical infection, omphalophlebitis, omphaloarteritis, and omphalourachitis.[35] Blood samples (heparin, EDTA, and serum) were collected

TABLE 4 Clinical, laboratory, and combined multivariable regression models for factors associated with mortality in 230 critically ill calves

Variable		Mortality	OR	95% CI	*P*-value	Se (%)	Sp (%)	Acc (%)
Clinical model								
n = 116	Gender (male)	70.3% (45/64)	4.3	1.5-11.9	.01	85.9	66.7	78.4
	Temperature (/°C increase)		0.55	0.36-0.85	.01			
	Color mucosae (pale)	84.4% (27/32)	7.1	1.9-26.2	.003			
	Abnormal behavior	77.1% (54/70)	3.3	1.3-8.5	.01			
Laboratory model								
Model A	Blood culture positive	79.3% (46/58)	3.3	1.6-7.0	.002	60.6	71.0	64.6
n = 178	Acidosis (≤7.18)	78.7% (37/47)	2.9	1.3-6.4	.01			
Model B	Hypoglycemia (≤55 mg/dL)	87.5% (35/40)	5.2	1.9-14.4	.001	54.3	84.1	65.5
n = 168	Acidosis (≤7.18)	78.7% (37/47)	3.5	1.4-8.8	.01			
Combined (clinical + laboratory) model								
Model A	Abnormal behavior	75.0% (48/64)	3.3	1.1-10.0	.03	85.7	73.2	80.8
n = 104	Gender (male)	68.3% (41/60)	4.3	1.4-13.2	.01			
	Tachycardia (>126 bpm)	67.4% (29/43)	3.4	1.1-10.8	.04			
	Color mucosae (pale)	84.6% (22/26)	10.4	2.5-43.7	.001			
	Acidosis (≤7.18)	86.2% (25/29)	5.3	1.3-21.9	.02			
	Blood culture positive	80.0% (28/35)	6.9	1.9-25.0	.003			
Model B	Abnormal behavior	73.1% (49/67)	3.1	1.2-8.0	.02	78.6	70.3	73.6
n = 106	Tachycardia (>126 bpm)	69.0% (29/42)	2.7	1.0-7.2	.05			
	Acidosis (≤7.18)	85.7% (24/28)	4.2	1.2-15.0	.03			
	Hypoglycemia (≤55 mg/dL)	92.0% (23/25)	10.4	2.1-51.5	.004			

Note: Abnormal behavior included an apathic state, decubitus, and/or neurological signs.
Abbreviations: 95% CI, 95% confidence interval; Acc, accuracy; n, calves in model; N, calves with variable present; OR, odds ratio; Se, sensitivity; Sp, specificity.

from the jugular vein using a vacutainer system (Venoject, Terumo, Leuven, Belgium) for blood gas analysis (RAPIDPoint 405 Siemens Healthcare, Beersel, Belgium), hematology (IDEXX ProCyte Dx Hematology Analyzer, DEXX Europe B.V., Hoofddorp, The Netherlands) and biochemical analysis (IDEXX Catalyst One Chemistry Analyzer, IDEXX Europe B.V., Hoofddorp, The Netherlands). History about previous antimicrobial treatment from the owner or local veterinarian was recorded in the worksheet.

2.4 | Blood culture and bacteriology

Blood was taken from the jugular vein after clipping, scrubbing with chlorhexidine, and disinfection with ethanol or isopropanol for aseptic preparation. Samples were taken with a 21G needle and 10 to 20 mL syringe by an operator wearing sterile gloves. Aerobic enriched media were used for sampling; BACTEC Plus Aerobic medium 8 to 10 mL, BD BACTEC Peds Plus medium 1 to 3 mL (BD, Erembodegem, Belgium) or both. Blood cultures were aerobically incubated at 35°C in an automated system for detection of microbial growth (BACTEC FX). Samples were considered negative after >5 days without growth in the BACTEC FX system. During the study, bacterial isolates were identified at 1 of 3 different laboratories using conventional (biochemical) identification methods, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or both. The disk diffusion method was used for antimicrobial susceptibility testing and interpreted according to either Clinical and Laboratory Standards Institute standards (CLSI),[36] European committee on antimicrobial susceptibility testing (EUCAST)[37] or Comité de l'antibiogramme de la Société Française de Microbiologie (CASFM).[38] Susceptibility results of fastidious organisms (e.g., *Corynebacterium* spp., *Trueperella pyogenes*) using the disk diffusion method were considered unreliable because only broth microdilution methods should be used to interpret susceptibility testing results for these bacteria.[39]

2.5 | Statistical analysis

Data were collected in a worksheet (Excel, Microsoft Inc, Washington) and transferred to a statistics program (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) for descriptive and statistical analysis. Binary logistic regression was used to determine risk factors for mortality. Three separate models were built: 1 including only clinical signs, a second using only blood variables and a third combining both. For each model, all factors first were tested univariably. Continuous factors were tested continuously as well as

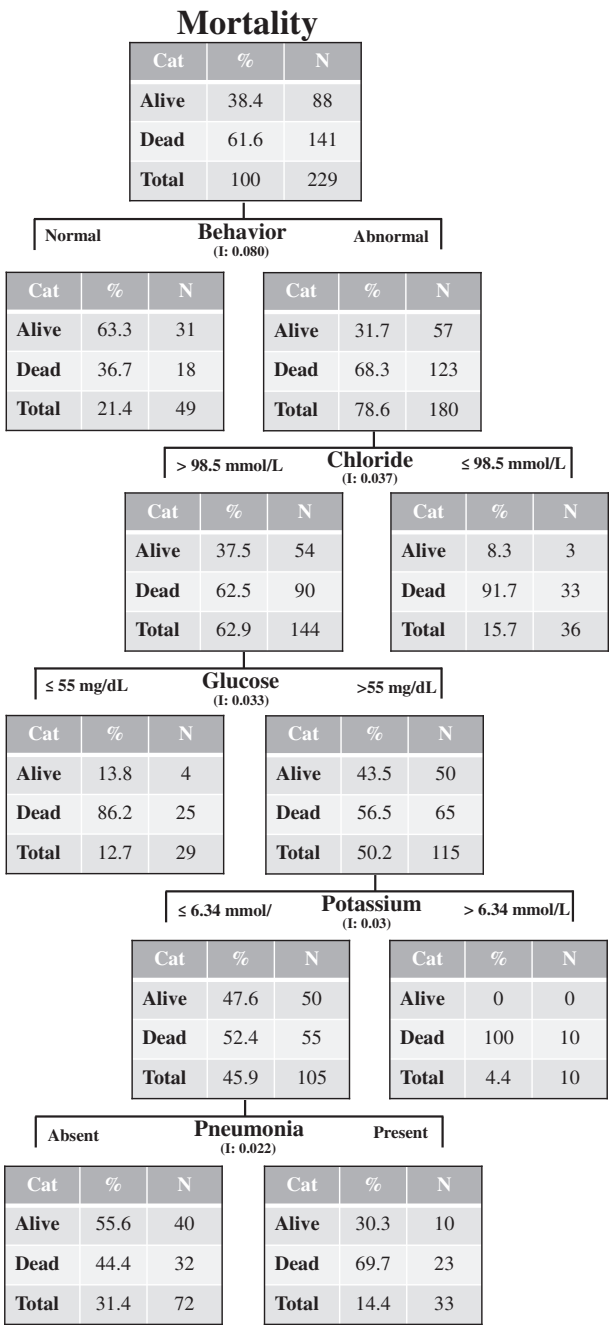

FIGURE 1 Decision tree for mortality in 229 critically ill calves based on clinical and laboratory findings. Cat, Category; I, improvement; N, calves included. Abnormal behavior included an apathic state, decubitus and/or neurological signs.

categorically, based on deviations from normal reference values,[40] quartiles, and binaries using a cut-off as determined by receiver operating characteristics curve analysis (ROC). Categorical factors with ≤20 observations were merged and recorded into broader categories or excluded if doing so was not possible and no final model could be generated otherwise. All factors with $P < .2$ were retained in the multivariable model, which was built backwards stepwise, gradually excluding nonsignificant predictors. Correlations between significant continuous factors were tested and, when >0.6, only the most significant factor was used for further modeling. Similarly, for categorical factors, associations were tested by Chi-square testing and logistic regression. When a significant association was found, variables were not withheld in the same model. Biologically plausible interactions were tested. Model fit was evaluated using the Hosmer-Lemeshow test for logistic models. Overall model fit was determined by accuracy. Significance was set at $P \leq .05$.

In a second approach, a classification and regression tree analysis (CART) was performed using SPSS (IBM). The CART method attempts to continuously split the data with maximum homogeneity within the node until homogeneity or imposed stopping criteria are met in a node.[41] The degree of nonhomogeneous subset in a node is an indication of impurity.[42] This impurity is measured by the Gini index, whereby the minimum of decrease in impurity was set at 0.0001. Growth limit was set at 20 observations in the parent node and 10 in the child node whereby the maximum depth of the decision tree was determined automatically. Missing values were excluded from the tree-growing process, because the number of surrogates was preset as 0, resulting in no surrogates or alternative predictors in the development of the decision trees. For validation of the tree, cross-validation was selected because of the small sample size.[42] Tree performance was evaluated based on diagnostic accuracy, sensitivity and specificity, positive (PPV) and negative (NPV) predictive value, and area under the curve (AUC). Both logistic regression and tree analysis models were solely explanatory models. Sample size was too small to have a training and validation data set of sufficient size for predictive modeling.

Finally, a survival analysis was performed. A Cox proportional hazards model was built with mortality as the outcome. The time until discharge or death was defined as survival time and mortality as the event (0/1). Right censoring was done at the day animals left the clinic. Time was calculated by subtracting the date of blood culture (inclusion) from the date of death or discharge from the clinic. Similar to logistic regression, a stepwise backwards approach was used, where variables that had univariable significance ($P < .05$) or $P < .2$ were included in the 3 multivariable models after correlation was excluded. Visualization of significant factors of interest was done using Kaplan-Meier survival curves.

3 | RESULTS

3.1 | Animals, diseases, and mortality risk

Our study population consisted of 230 critically ill calves. Mean ± SD and median age were 15.4 ± 22.6 and 8 days, respectively (range [R], 0-129). Of the calves, 87% (199/230) were <1 month of age. Most calves (81.7%, n = 188) belonged to the Belgian Blue (BB) beef breed, 8.7% (n = 20) were Holstein Friesian (HF), 3.9% (n = 9) Blonde d'Aquitaine, 1.7% (n = 4) Maine Anjou, 1.7% (n = 4) crossbreds, 1.3% (n = 3) other breeds, and 0.9% (n = 2) were of unknown breed. There were slightly more male (n = 112) than female (n = 106) calves and sex was not recorded for 12 calves. Mean ± SD and median rectal temperature were 38.8°C ± 1.53°C and 38.9°C, respectively (R, unmeasurably low-41.9°C). Mean and median heart rate were

TABLE 5 Clinical, laboratory, and combined multivariable survival analysis for factors associated with mortality in 230 critically ill calves

Variable		HR	95% CI	*P*-value
Clinical model				
n = 128	Temperature (/°C increase)	0.82	0.71-0.94	.005
	Abnormal behavior	2.4	1.3-4.2	.003
Laboratory model				
Model A	Blood culture positive	1.9	1.3-2.7	.002
n = 178	Acidosis (≤7.18)	1.6	1.0-2.3	.03
Model B	Hypoglycemia (≤55 mg/dL)	2.1	1.4-3.2	<.001
n = 168	Acidosis (≤7.18)	1.7	1.1-2.5	.01
Combined (clinical + laboratory) model				
Model A n = 128	Abnormal behavior	2.3	1.3-4.0	.005
	Temperature (/°C increase)	0.82	0.72-0.95	.01
	Blood culture positive	1.6	1.0-2.5	.05
Model B n = 175	Temperature (/°C increase)	0.87	0.78-0.96	.004
	Hypoglycemia (≤55 mg/dL)	2.2	1.5-3.3	<.001

Note: Abnormal behavior included an apathic state, decubitus, and/or neurological signs.
Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio.

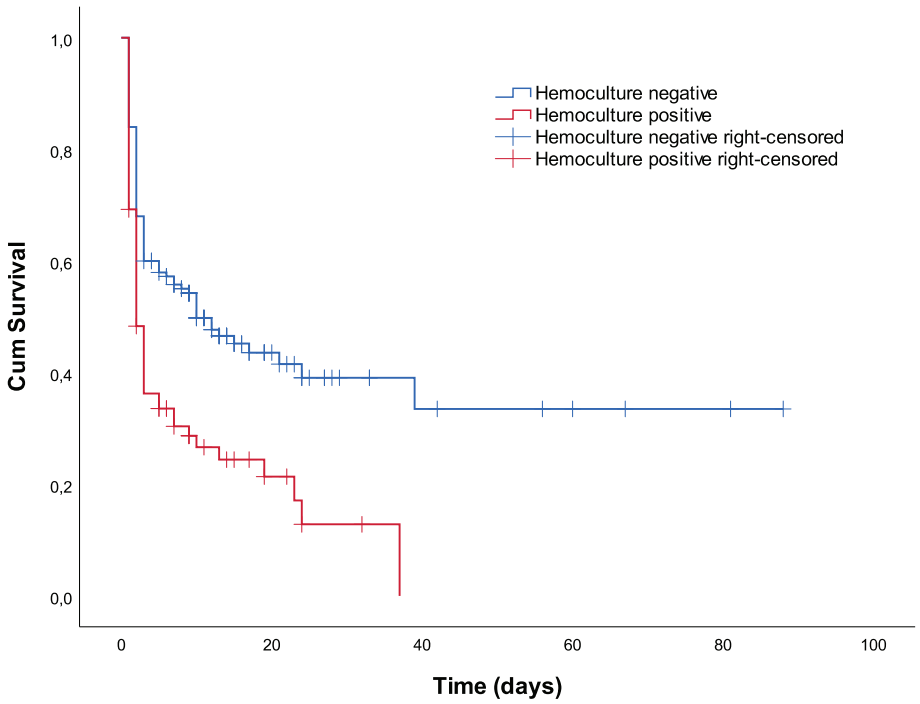

FIGURE 2 Survival graph for mortality of critically ill calves with or without positive blood culture (228 cases; 2017-2020; Log Rank test: χ^2 13.4; $df = 1$; $P < .001$)

122.2 ± 33.3 and 120 beats/min (R, 48-220) and these values were 61.4 ± 33.8 and 52 breaths/min (R, 10-156) for respiratory rate.

An overview of clinical diagnosis in relation to sepsis and mortality risk is given in Table 1. Enteritis and diarrhea (51.7%) and pneumonia (45.7%) were diagnosed most frequently, followed by neonatal respiratory distress syndrome (NRDS, 17.8%) and omphalitis (17.8%). In 19 calves, the final diagnosis was either unknown or not recorded. In 34.3% (79/230) of the calves, sepsis was confirmed by positive blood culture. Mortality risk was significantly higher in calves with sepsis than in non-septic calves (76.9% [60/78] vs 53.6% [81/151]; odds ratio [OR], 2.9; 95% confidence interval [CI]: 1.6-5.3; $P = .001$) as shown in Table 2. Of the calves with sepsis, 44.3% (35/79) had enteritis, 51.9% had pneumonia (41/79), 11.4% (9/79) omphalitis, 7.6% (6/79) arthritis, and 11.4% (9/79) had NRDS. Comorbidities (Table 1) were frequent (54.8% [126/230]), but a comorbidity did not significantly increase the risk of mortality (OR: 0.93; 95% CI: 0.54-1.6; $P = .79$), or the risk of sepsis (OR: 0.91; 95% CI: 0.52-1.6; $P = .72$).

In total, 61.3% (n = 141) of the calves died, and in 1 case information about mortality was absent. Mean ± SD and median survival time in all cases was 9.2 ± 12.5 and 5, respectively (R, 1-88). Mean ± SD and

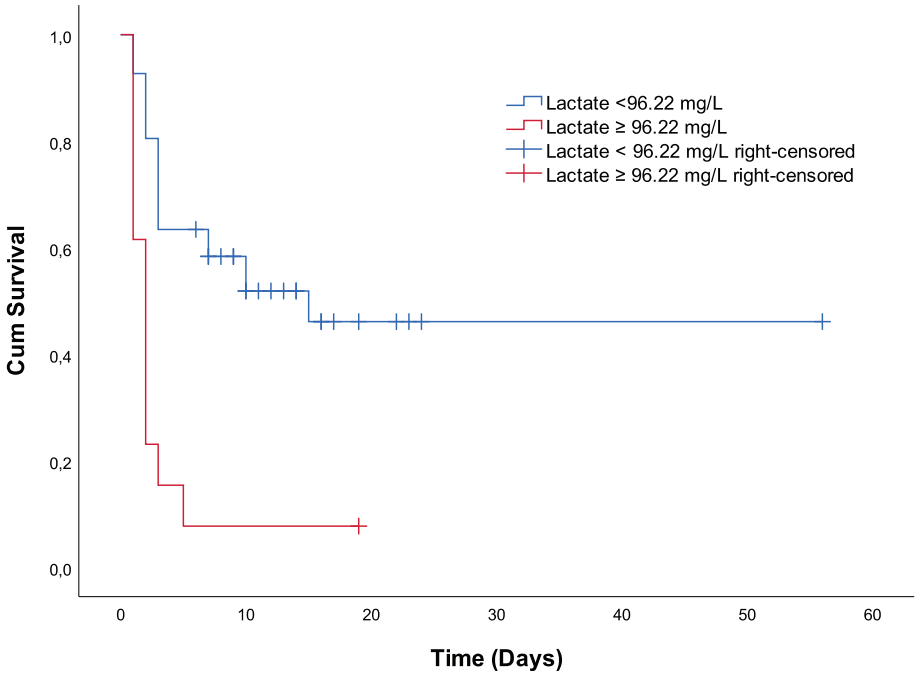

FIGURE 3 Survival graph for mortality of critically ill calves with or without lactate ≥96.22 mg/L (54 cases; 2017-2020; Log Rank test: χ^2 12.2; $df = 1$; $P < .001$)

median survival time in the animals discharged from the clinic was 17.1 ± 15.7 and 13, respectively (R, 1-88). Mean ± SD and median survival time in the animals that died was 4.3 ± 6.3 and 2, respectively (R, 1-39).

3.2 | Risk factors for mortality in critically ill calves

Tables 2 and 3 present associations with mortality in critically ill calves for clinical and laboratory variables, respectively. Univariable analysis showed that septicemic, dehydrated, or hypothermic calves with either metabolic or respiratory acidosis were more likely to die, as well as calves with hypoglycemia or pale mucous membranes. Several factors were significantly correlated with skin turgor (dehydration, hematocrit, and serum sodium, urea, and creatinine concentrations), and skin turgor was selected for the multivariable model. Acidosis was highly correlated with partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-) concentration, base excess (BE), and serum potassium concentration, and thus pH was included in the final model. Positive blood culture was significantly correlated with hypoglycemia. Because both factors were relevant, both multivariable logistic models (1 with sepsis [A] and 1 with blood glucose [B]) are shown in Table 4. The final combined model including blood culture (Model A) had the highest accuracy, but in model B results are more rapidly available to the practitioner because it contains cow-side tests.

The results of the CART analyses with clinical and laboratory factors are found in Figure 1. The tree including history and clinical variables (not shown) consisted of behavior (abnormal), followed by mucous membranes (pale), temperature (≤37.2°C), diarrhea (absent), and breed (BB). Its accuracy was 69.9%, with a sensitivity of 83.0% and specificity of 48.9%. The AUC was 0.659 and PPV and NPV were 72.2% and 64.2%, respectively. The combined (clinical findings and blood test results) tree consisted of behavior; serum chloride, glucose, and potassium concentrations and pneumonia in consecutive order. This tree was 70.7% accurate, and had sensitivity and specificity of 64.5% and 80.7%, respectively. The AUC was 0.715, and the PPV and NPV were 84.3% and 58.6%, respectively.

Results of the survival analysis are shown in Table 5. In the univariable survival analysis, the same risk factors as in logistic regression had $P < .20$ and therefore were included in the multivariable model, with the exception of breed and tachycardia (data of univariable analysis not shown). Figure 2 shows survival in critically ill calves stratified based on blood culture test results (positive vs negative). Lactate was significant in the univariable analysis, but not included in the multivariable model because of the limited number of observations (Figure 3).

3.3 | Bacteria and antimicrobial resistance

Table 6 provides an overview of the most frequently isolated bacteria and their respective antimicrobial susceptibility. Blood culture was positive in 79 cases, but in only 67 animals were ≥1 bacteria identified to species level. In 4 blood cultures, >1 bacterial species was isolated: (1) *Escherichia coli* and *Streptococcus pluranimalium*, (2) *Klebsiella pneumoniae* and *Streptococcus pluranimalium*, (3) *Bacillus pumilus*, *S. pluranimalium*, and *Bibersteinia trehalosi*, and (4) *Enterococcus* sp. and *Staphylococcus sciuri*. In 12 cases, after a positive result from the BACTEC FX, no bacteria were isolated either as a result of human error (clinician or laboratory) or because involved bacteria did not grow on the standard media used after enrichment medium, thus resulting in a total of 72 bacterial isolates.

TABLE 6 Antimicrobial resistance for the isolated bacteria out of 79 blood cultures of critically ill calves

Bacteria (Number of isolates)	% AMR (n)																
	PEN	AMP/AMX	AMC	LEX	CFT	CFQ	MACR	LIN	FFC	SPT	GEN	FMQ	ENR	TET	DOX	SXT	% MDR
Escherichia coli (22)	*100 (22/22)*[a]	95.5 (21/22)	42.9 (9/21)	29.4 (5/17)	28.6 (6/21)	27.3 (6/22)	*100 (22/22)*[a]	*100 (22/22)*[a]	52.9 (9/17)	10.5 (2/19)	31.8 (7/22)	42.1 (8/19)	22.7 (5/22)	90.9 (20/22)	80.9 (17/21)	77.3 (17/22)	90.9 (20/22)
***Staphylococcus* sp.** (11) *S.* sp. (5) *S. xylosis* (3) *S. sciuri* (2) *S. chromogenes* (1)	55.6 (5/9)	55.6 (5/9)	44.4 (4/9)	44.4 (4/9)	44.4 (4/9)	44.4 (4/9)	33.3 (2/6)	80.0 (4/5)	50.0 (3/6)	50.0 (1/2)	50.0 (1/2)	0 (0/2)	14.3 (1/7)	75.0 (6/8)	55.6 (5/9)	100 (0/9)	55.5 (5/9)
***Salmonella* sp.** (7)	*100* (7/7)[a]	50.0 (3/6)	16.7 (1/6)	0 (0/4) *100* (7/7)[b]	0 (0/5)	0 (0/4)	*100* (7/7)[a]	*100* (7/7)[a]	0 (0/4)	0 (0/4) *100* (7/7)[b]	0 (0/6) *100* (7/7)[b]	0 (0/5)	0 (0/6)	0 (0/5)	0 (0/5)	33.3 (2/6)	0 (0/6)
Other Enterobacteriaceae (7) *Klebsiella* sp. (2) *Raoultella* sp. (2) *Enterobacter* sp. (2) *Coliforms* sp. (1)	*100* (7/7)[a]	100 (1/1) *100 (6/6)*[a]	50.0 (2/4) *100 (2/2)*[a]	33.3 (1/3) *100 (2/2)*[a]	40.0 (2/5)	20.0 (1/5)	*100* (7/7)[a]	*100* (7/7)[a]	40.0 (2/5)	25.0 (1/4)	66.7 (4/6)	50.0 (3/6)	16.7 (1/6)	80.0 (4/5)	80.0 (4/5)	83.3 (5/6)	66.7 (4/6)
***Bacillus* sp.** (4) *B. licheniformis* (1) *B. cereus* (1) *B. pumilus* (1) *B.* sp. (1)	50.0 (1/2) *100 (1/1)*[a]	0 (0/2) *100 (1/1)*[a]	0 (0/2)	100 (1/1) *100 (1/1)*[a]	100 (2/2) *100 (1/1)*[a]	100 (2/2) *100 (1/1)*[a]	0 (0/3)	50.0 (1/2)	0 (0/2)	/	0 (0/1)	/	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)
***Streptococcus* sp.** (4) *S. pluranimalium* (3) *S. uberis* (1)	50.0 (1/2)	33.3 (1/3)	0 (0/2)	0 (0/2)	0 (0/3)	0 (0/3)	100 (2/2)	100 (2/2)	0 (0/2)	100 (1/1)	100 (1/1)	*100 (4/4)*[a]	33.3 (1/3)	100 (3/3)	100 (3/3)	100 (3/3)	100 (3/3)
Other bacteria (17)	50.0 (3/5) *100 (2/2)*[a]	20.0 (2/10)	16.7 (1/6)	40.0 (4/10)	20.0 (2/10)	20.0 (2/10)	50.0 (2/4) *100 (1/1)*[a]	100 (3/3) *100 (1/1)*[a]	0 (0/10)	16.7 (1/6)	25.0 (1/4)	16.7 (1/6)	33.3 (3/9)	50.0 (5/10) *100 (1/1)*[a]	20.0 (2/10)	30.0 (3/10)	30.0 (3/10)

Abbreviations: AMC, amoxicillin clavulanic acid; AMP, ampicillin; AMX, amoxicillin; CFQ, cefquinome; CFT, ceftiofur; DOX, doxycycline; ENR, enrofloxacin; FFC, florfenicol; FMQ, flumequine; GEN, gentamicin; LEX, cephalexin; LIN, lincosamides; MACR, macrolides; MDR, multidrug resistance; PEN, penicillin; SPT, spectinomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracyclines.

[a]Intrinsic resistance: EUCAST(2021),[71] CLSI M100-ED32 (2022)[72] and WHO (1997).[73]

[b]In vivo resistance: CLSI M100-Ed31 (2021).[74]

TABLE 7 Theoretical inappropriateness per antimicrobial for the isolated bacteria of 79 blood cultures of critically ill calves

Antimicrobial (number isolates with known susceptibility)	Acquired resistance % (number)	Intrinsic resistance % (number)	Unclear mechanism % (number)	Inappropriate treatment[a] % (number)
Penicillin (57)	20.4 (10)	79.6 (39)	/	86.0 (49)
Ampicillin/amoxicillin (60)	82.5 (33)	17.5 (7)	/	66.7 (40)
Amoxicillin + clav. acid (52)	89.5 (17)	10.5 (2)	/	36.5 (19)
Cefalexin (52)	56.0 (14)	40.0 (10)	4.0 (1)	48.1 (25)
Ceftiofur (56)	88.2 (15)	5.9 (1)	5.9 (1)	30.4 (17)
Cefquinome (56)	87.5 (14)	6.3 (1)	6.3 (1)	28.6 (16)
Macrolides (52)	14.0 (6)	86.0 (37)	/	82.7 (43)
Lincosamides (49)	21.3 (10)	78.7 (37)	/	95.9 (47)
Florfenicol (46)	100 (14)	/	/	30.4 (14)
Spectinomycin (39)	46.2 (6)	53.8 (7)	/	33.3 (13)
Gentamicin (43)	66.7 (14)	33.3 (7)	/	48.8 (21)
Flumequine (42)	75.0 (12)	25.0 (4)	/	38.1 (16)
Enrofloxacin (56)	100 (11)	/	/	19.6 (11)
Tetracycline (57)	97.4 (38)	2.6 (1)	/	68.4 (39)
Doxycycline (56)	100 (31)	/	/	55.4 (31)
Potentiated sulphonamides (59)	100 (30)	/	/	50.8 (30)

[a]Appropriateness based on antimicrobial sensitivity results and intrinsic resistance, displaying whether the antimicrobial would have been appropriate if therapy was initialized with that particular drug.

In total 62.5%, (45/72) Gram-negative and 37.5% (27/72) Gram-positive isolates were obtained. Bacteria isolated from blood culture, not listed in Table 6 because of low isolation frequency included; *Enterococcus* sp. (1), *Mannheimia haemolytica* (1), *Trueperella pyogenes* (1), *Bibersteinia trehalosi* (1), *Campylobacter jejuni* (1), *Serratia ureilytica* (1), *Chryseobacterium hominis* (1), *Microbacterium lacticum* (1), *Globicatella* sp. (1), *Myroides odoratimimus* (1), *Proteus mirabilis* (1), *Paenibacillus amylolyticus* (1), and unidentified coryneforms (1), Gram-negative Cocci (1), Gram-positive cocci (1), Gram-positive rods (1), and Gram-negative rods (1). The most frequently isolated species was *E. coli* 30.6% (22/72). Other Enterobacteriaceae, including *Salmonella* sp., *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Raoultella ornithinolytica*, coliforms, and *Enterobacter cloacae*, also were prevalent (19.4%, 14/72). The most frequently isolated Gram-positive genus was *Staphylococcus* spp. (15.2%, 11/72). Of the isolated staphylococci, 4 were methicillin-resistant and 1 produced beta-lactamase. Multidrug resistance (MDR) according to bacterial genus and species also is presented in Table 6. Especially for *E. coli* (90.5%), the prevalence of MDR isolates was high. Overall, 59.3% (35/59) of the bacteria with available susceptibility data featured MDR.

Table 7 presents the antimicrobial resistance and theoretical appropriateness of the isolates by antimicrobial class, including intrinsic resistance. Theoretically inappropriate treatment in frequently used (first intention) antimicrobials such as penicillin (86.0%), SXT (50.8%), and aminopenicillins (66.7%) was high, whereas resistance was lower, but still noteworthy for the critically important antimicrobials (CIA) ceftiofur (30.4%), cefquinome (28.6%), and enrofloxacin (19.1%).

In the 79 sepsis cases, clinicians most often opted for combination treatment of enrofloxacin and penicillin (40). This combination was only inappropriate in 12.1% (4/33) of the bacteria for which it could be determined. Other combination treatments included gentamicin-penicillin (3), SXT-enrofloxacin (2), enrofloxacin-ampicillin (1), SXT-enrofloxacin-penicillin (1), SXT-gentamicin-penicillin (1), and lincomycin-spectinomycin-penicillin (1). In some cases, the combination of the antimicrobials was not immediately administered, but was given subsequently on the same day, because of lack of clinical improvement or rapid deterioration of the treated calf. In a few cases, monotherapy with enrofloxacin (5), amoxicillin (4), ceftiofur (3), amoxicillin clavulanic acid (2), and penicillin (2) was administered. Three antimicrobials were only given once (SXT, oxytetracycline, neomycin-penicillin). For 11 calves, no records about antimicrobial treatment were available. Observed appropriateness per case could be determined in 48 animals. Treatment per case (taking combination therapy into account) was appropriate in 79.1% (38/48) of the cases. Observed inappropriate treatment did not significantly affect mortality risk (64.9% appropriate vs 70.0% inappropriate treatment; OR: 1.3; 95.0% CI: 0.28-5.7; $P = .76$). Also, the use of CIA (quinolones and cephalosporins) did not improve survival (OR: 1.0; 95.0% CI: 0.28-3.8; $P = .97$).

4 | DISCUSSION

Different diseases can result in critical illness in calves. As shown in our study, sepsis is very frequent (34.3%) in this population and increases mortality risk. As expected, calves with enteritis, NRDS, and pneumonia can present as critically ill, but only pneumonia significantly increased the odds of mortality.

In our study, general mortality in these critically ill calves (61.3%) was higher than previously reported in a German veterinary teaching hospital (22%).[28] One explanation might be that Belgian farmers and veterinarians refer animals at a more advanced disease stage, thereby hampering survival chances. This hypothesis is substantiated by the fact that nearly 18% of the calves were hypothermic at presentation. Hypothermia is highly associated with mortality both in our study as well as in human medicine, and results in worse Sequential Organ Failure Assessment (SOFA) scores in patients with sepsis.[43,44] Humans with septic shock also have mortality of up to 60% to 80%.[45] Differences in clinician experience or in-house protocols between both clinics might be another explanation. However, the most likely explanation for the high mortality is the large proportion of BB cattle in our study. In the final multivariable model, breed was not significant. The BB breed however is known to be very susceptible to diseases, especially those affecting the respiratory system.[46] A high percentage of calves in our study had respiratory disease. Bovine respiratory disease is associated with an increased hazard for mortality in calves.[47] Increased mortality risk is seen in calves with lung consolidation ≥6 cm in depth on lung ultrasound examination.[48] In the population in our study, mortality risk even increased with consolidation ≥1 cm in depth. Also, a substantial proportion of critically ill calves suffered from NRDS, which is in BB cattle linked with low surfactant protein C.[30] Lung ultrasound examination in NRDS calves often shows diffuse B-lines, indicating an interstitial lung pattern (i.e., lung edema). However, other causes of edema (e.g., heart defects) should be excluded. In human medicine, chest radiography is preferred, but lung ultrasound examination is seen as a valuable complementary diagnostic tool,[49] and can be utilized in cattle as a cow-side test.[50]

The high mortality in these calves remains remarkable, emphasizing the need for prognostic information to make a rational economic decision about treatment. Our study aimed at identifying risk factors for mortality that can be useful for this purpose. Regarding clinical variables associated with mortality in calves, hypothermia, pale mucous membranes, and abnormal behavior appear most promising, because they were significant in logistic regression, survival analysis, and included in clinical tree analysis. In humans, mental state also is considered an important predictive factor for survival in critically ill patients (eg, Glasgow Coma Score).[44] Clinically abnormal behavior in calves often is associated with acidosis (D-lactatemia), hypoglycemia, and hypercapnia,[28,51] which were all significantly linked with mortality in our study. Acidemia and hypoglycemia were included in 1 or both final mortality models. Other promising significant laboratory variables were hyperkalemia, hemoconcentration, and L-lactatemia, but these were not selected for the multivariable model-building process because of correlation with pH and dehydration or because of insufficient observations (e.g., lactate).[26-28] Acidosis, as well as glucose and lactate concentrations, appeared most promising as cow-side tests, and should be further explored. In human medicine, as well as in diarrheic calves, hyperchloremia appears to predict mortality.[27,52] In our study, hypochloremia was included in the decision tree. A possible explanation may be that hypochloremia often is associated with ileus in cattle.[53] Ileus is associated with abdominal emergencies, which is a known risk factor for mortality.[28] Our study population had a limited number of arthritis cases, making it impossible to evaluate its contribution to mortality. However, previous studies in critically ill and veal calves identified arthritis as an important contributor to mortality.[28,47] It was remarkable that none of the comorbidities increased mortality risk in calves, contrary to what is observed in human medicine.[54]

When evaluating the different mortality models, it is important to acknowledge that these are explanatory models, that try to identify which factors contribute to mortality. It was not our objective to develop a predictive tool for mortality, because our sample size was too small to provide a training and validation data set of sufficient size. Overall, the best-performing model was the logistic regression combined model A (Table 4). However, although high accuracy is desirable, practical applicability also is important and models containing immediately determinable variables (e.g., clinical or cow-side tests) are more likely to be used in practice. Hence, the clinical logistic regression model, with almost equivalent accuracy to the combined model, should be considered for cost-benefit and pragmatic reasons. However, a main reason for predicting mortality is that in animals with very unfavorable prognosis, the client might opt for euthanasia instead of treatment. In this context, high specificity is desirable, because high PPV is needed so as not to recommend euthanasia unnecessarily. Specificity was higher in the combined clinical and laboratory model. The combined clinical and laboratory tree had the highest specificity, but was less accurate. The risk factors in the combination tree and survival analysis might have the highest external validity, because with the exception of behavior, only objectively measurable factors were involved. In conclusion, several clinical and laboratory variables showed promising predictive possibilities for mortality, with sepsis among them. To our knowledge, our study is the first observational study demonstrating increased mortality risk in calves with sepsis, a contested topic in human medicine.[55-61]

Information on bacteria involved in sepsis and their antimicrobial resistance profile is limited, especially in Europe. Therefore, our second objective was to obtain knowledge on the bacteria involved in sepsis in critically ill calves and their antimicrobial susceptibility. Gram-negative bacteria predominated, but the difference with respect to Gram-positive bacteria was not as large as previously reported.[62] As in previous studies, *E. coli* was most prevalent,[62] and often showed MDR. The most rational way to use antimicrobials for sepsis in calves, and to maximize survival, is likely the same as in humans, namely de-escalation.[10,12,13,63] With this concept, treatment is started with IV administration of a broad-spectrum bactericidal antimicrobial. As soon as antimicrobial susceptibility test results are available, a narrow-spectrum antimicrobial can be selected, limiting selection pressure on commensals.[10,63] Administration of an appropriate antimicrobial early in the disease course is crucial for survival.[12] However, in our study, this association could not be made in calves, likely because of limited power.

In a clinical setting, animals often are submitted after treatment failure by the ambulatory veterinarian. Therefore, it is plausible that, provided appropriate sampling, antimicrobials with a broader working spectrum such as CIA, are used more often in referral institutes. This

also is seen in our clinic with the use of fluoroquinolones. Nevertheless, a critical determination of whether the animal needs CIA remains indispensable. Based on our study results, SXT or aminopenicillins would have been appropriate in only 49.2% and 33.3% of the cases, respectively. In contrast, fluoroquinolones and cephalosporins both would have been appropriate in approximately 80% to 70% of the cases. In our clinic, penicillin often is added to extend the spectrum of fluoroquinolones. The frequent inappropriate use of penicillin (86%) against aerobes in our study suggests that it is not systematically necessary. However, penicillin is known to be effective against anaerobes, which were not included in our study. In addition, a meaningful decrease in inappropriate usage is seen if enrofloxacin monotherapy (19.1%) is compared with enrofloxacin-penicillin (12.1%). Overall, the prevalence of bacteremia in this population of critically ill calves and their high frequency of resistance against current primary treatment products might be seen as a justification for the IV use of broad spectrum, bactericidal antimicrobials, and potentially CIA. On the other hand, it might be reasoned that 34% sepsis prevalence is too low for standardized use of CIA in critically ill calves, and additional tests to identify sepsis should be considered.

Our study had some limitations. Its retrospective nature is an important limitation for both the bacteriology as well as for the risk factors associated with mortality. Poorly documented clinical factors and historical information about earlier treatment by an ambulatory veterinarian hamper the integrity of the data. In addition, the description and interpretation of clinical factors are susceptible to interobserver and intraobserver bias. The latter is partly countered by clinician training provided by the same senior supervisor. Nonetheless, we sought to divide the factors into categories that could be objectively differentiated when constructing the database. Failure to identify certain bacterial isolates also resulted in a substantial loss of data. As a result of using outsourced data for bacterial identification and susceptibility testing, the reliability of these data cannot be assured. Nonetheless, withholding the antimicrobial data also was not desirable because our study represented a first attempt to display regional resistance data. Regrettably, for economic reasons, we did not include selective media for anaerobic, fungal, or yeast infections. In humans, *Candida* sp. is responsible for approximately 5% of all cases of severe sepsis and septic shock and constitutes the fourth most common hematogenous infection.[64] Likely, these type of infections are more common in the intensive care setting, and their role in calf sepsis on farm remains to be determined. Considering the antimicrobial susceptibility testing, there are several important limitations. The first is the use of disk diffusion instead of broth microdilution, which is regarded as the gold standard for antimicrobial susceptibility testing, especially for fastidious organisms. Considerable disk diffusion data were missing, and not all data were reliable to interpret. However, disk diffusion is considered a cost-effective method for most bacterial species and is used routinely in many veterinary and human medical laboratories.[65-67] Nonetheless, the lack of raw antimicrobial susceptibility data (diameter) affects reliability in our study because several laboratories were involved throughout the study period, resulting in the use of different (and sometimes inappropriate) antibiotic testing panels, and in the use of different standards and associated clinical breakpoints to interpret the antibiograms. Currently presented bacteriological data provide a first assessment of the antimicrobial resistance in bacteria causing sepsis in calves. Care should be exercised, however, in extrapolating these results for the abovementioned reasons. In addition, these bacteria were isolated at a single university clinic in a single country, and might not be representative for septicemic calves elsewhere. More data, taking the limitations of the current dataset into account, are needed to validate these observations.

As a last limitation, we were confronted by a lack of information on clinical recognition of septicemic calves. Although recent studies on the hemodynamic aspects of sepsis and its treatment exist,[68,69] these were not within the scope of our study. The definition of sepsis in former articles relied on the combination of systemic inflammatory response syndrome (SIRS) and the suspicion of infection. Nevertheless, SIRS was not taken as a criterion in our article, because SIRS also was dismissed as sepsis criterion in human medicine because of its lack in specificity and sensitivity.[1] Our study showed that a positive blood culture is linked to increased mortality and therefore should not be ignored when trying to define sepsis. Also in human medicine, blood culture remains the gold standard and primary tool for detecting bloodstream infections[70] and is a more objective tool to help define sepsis, compared to focusing only on clinical signs. Nonetheless, blood cultures are not 100% sensitive, even when using a larger volume of blood.[70] Use of more modern and enriched blood culture media in our study did not result in more positive blood cultures compared to previous studies.[6,7] However, we believe the prevalence of sepsis in our study likely was underestimated, because a number of calves received antimicrobials before referral. Hence, the bacteria might already have been removed from the blood, without resolution of clinical signs. Both for research purposes and for veterinary practice, an accurate definition of sepsis in calves is needed to improve the quality of studies on predictive models, risk factors and biomarkers.

In conclusion, sepsis is frequent among critically ill calves and increases mortality risk. Mostly Gram-negative, but also Gram-positive bacteria are involved, necessitating the use of broad-spectrum antimicrobial treatment in the absence of rapid diagnostic testing. Antimicrobial resistance is frequent in isolates from calves with sepsis and, as a consequence, primary antimicrobial treatment is often inappropriate. Different clinical and laboratory risk factors for mortality in critically ill calves were identified that show potential for future research aiming at creating predictive models to assist farmers and veterinarians in their decision making process for critically ill calves, potentially cow-side, on the farm.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287
2. Pardon B, Deprez P. Rational antimicrobial therapy for sepsis in cattle in face of the new legislation on critically important antimicrobials. *Vlaams Diergeneeskd Tijdschr*. 2018;87(1):37-46.
3. Roy MF. Sepsis in adults and foals. *Vet Clin Equine*. 2004;20:41-61.
4. Montealegre F, Lyons BM. Fluid therapy in dogs and cats with sepsis. *Front Vet Sci*. 2021;8:622127. doi:10.3389/fvets.2021.622127
5. Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet Respir Med*. 2014;2(5):380-386. doi:10.1016/S2213-2600(14)70061-X
6. Fecteau G, Paré J, Van Metre DC, et al. Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. *Can Vet J*. 1997;38(2):101-104.
7. Lofstedt J, Dohoo IR, Duizer G. Model to predict septicemia in diarrheic calves. *J Vet Intern Med*. 1999b;13(2):81-88. doi:10.1892/0891-6640(1999)013<0081:Mtpsid>2.3.Co;2
8. Buckman SA, Turnbull IR, Mazuski JE. Empiric antibiotics for sepsis. *Surg Infect (Larchmt)*. 2018;19(2):147-154. doi:10.1089/sur.2017.282
9. Liang SY, Kumar A. Empiric antimicrobial therapy in severe sepsis and septic shock: optimizing pathogen clearance. *Curr Infect Dis Rep*. 2015;17(7):493. doi:10.1007/s11908-015-0493-6
10. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Crit Care Med*. 2017;45(3):486-552. doi:10.1097/ccm.0000000000002255
11. Davey PG, Marwick C. Appropriate vs. inappropriate antimicrobial therapy. *Clin Microbiol Infect*. 2008;14(3):15-21.
12. Kumar A, Ellis P, Arabi Y, et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest*. 2009;136(5):1237-1248. doi:10.1378/chest.09-0087
13. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med*. 2013;39(2):165-228. doi:10.1007/s00134-012-2769-8
14. EMA Committee for Medicinal Products for Veterinary Use (CVMP) and EFSA Panel on Biological Hazards (BIOHAZ), Murphy D, Ricci A, et al. EMA and EFSA joint scientific opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA). *EFSA J*. 2017;15:15. doi:10.2903/j.efsa.2017.4666
15. Regulation (EU) 2019/6 of the European Parliament and of the Council of December 11, 2018 on veterinary medicinal products and repealing Directive 2001/82/EC.
16. AMCRA. Formularium voor verantwoord gebruik van antibacteriële middelen in de rundveehouderij [Internet]; 2021. https://formularium.amcra.be/i/78 [In Dutch]. Accessed November 17, 2021.
17. Koninklijke Nederelandse Maatschappij voor Diergeneeskunde (KNMVD) Werkgroep voor veterinair antibioticumbeleid (Wvab). Formularium Vleeskalveren en Vleesvee; 2017. https://www.knmvd.nl/app/uploads/sites/4/2019/09/wvab-formularium-vleeskalveren-en-vleesvee_230919.pdf [In Dutch].
18. Koninklijk besluit July 21, 2016 Koninklijk besluit betreffende de voorwaarden voor het gebruik van geneesmiddelen door de dierenartsen en door de verantwoordelijken van de dieren. Belgisch Staatsblad, 46569-46586. [In Dutch/French].
19. Köper LM, Bode C, Bender A, Reimer I, Heberer T, Wallmann J. Eight years of sales surveillance of antimicrobials for veterinary use in Germany—what are the perceptions? *PLoS One*. 2020;15(8):e0237459. doi:10.1371/journal.pone.0237459
20. Autoriteit Diergeneesmiddelen (SDa). *Het gebruik van fluorochinolonen en derde en vierde generatie cefalosporines in landbouwhuisdieren*. Utrecht, the Netherlands: Rapportage van het SDa Expertpanel. 2013;1-24.
21. Delanghe JR, Speeckaert MM. Translational research and biomarkers in neonatal sepsis. *Clin Chim Acta*. 2015;451:46-64. doi:10.1016/j.cca.2015.01.031
22. Fecteau G, Van Metre DC, Pare J, et al. Bacteriological culture of blood from critically ill neonatal calves. *Can Vet J*. 1997;38(2):95-100.
23. Hariharan H, Bryenton J, St-Onge J, et al. Blood cultures from calves and foals. *Can Vet J*. 1992;33:56-57.
24. Aldridge BM, Garry FB, Adams R, et al. Neonatal septicemia in calves: 25 cases(1985-1990). *J Am Vet Med Assoc*. 1993;203:1324-1329.
25. Uetake K. Newborn calf welfare: a review focusing on mortality rates. *Anim Sci J*. 2013;84(2):101-105. doi:10.1111/asj.12019
26. Seifi H, Mohri M, Shoorei E, et al. Using haematological and serum biochemical findings as prognostic indicators in calf diarrhoea. *Comp Clin Pathol*. 2006;15:143-147. doi:10.1007/s00580-006-0620-8
27. Fayet JC, Overwater J. Prognosis of diarrhoea in the newborn calf: statistical analysis of blood biochemical data. *Ann Rech Vet*. 1978;9(1):55-61.
28. Trefz FM, Lorenz I, Lorch A, Constable PD. Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. *PLoS One*. 2017a;12:8.
29. Ostermann M, Sprigings D. The critically ill patient. In: Springings D, Chambers JB, eds. *Acute Medicine*. 5th ed. New Jersey, U.S: John Wiley & Sons Ltd. 2017;1-8.
30. Danlois F, Zaltash S, Johansson J, et al. Very low surfactant protein C contents in newborn Belgian white and blue calves with respiratory distress syndrome. *Biochem J*. 2000;351(3):779-787.
31. Yadav S, Lee B, Kamity R. *Neonatal Respiratory Distress Syndrome*. Treasure Island: StatPearls Publishing; 2021.
32. Meghani SH, Buck HG, Dickson VV, et al. The conceptualization and measurement of comorbidity: a review of the interprofessional discourse. *Nurs Res Pract*. 2013;2013:192782. doi:10.1155/2013/192782
33. Sweeney MT, Lubbers BV, Schwarz S, Watts JL. Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens—authors' response. *J Antimicrob Chemother*. 2018;74(2):536-537. doi:10.1093/jac/dky470
34. Rosenberger G. *Die klinische Untersuchung des Rindes*. 2th ed. Hamburg/Berlin: Verlag Paul Parey. 1977.
35. Wieland M, Mann S, Guard CL, Nydam DV. The influence of 3 different navel dips on calf health, growth performance, and umbilical

infection assessed by clinical and ultrasonographic examination. *J Dairy Sci*. 2017;100(1):513-524.
36. Clinical & Laboratory Standards Institute: CLSI VET08 ED4. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 4th ed.Wayne, PA: CLSI; 2018.
37. EUCAST: European Committee on Antimicrobial Susceptibility Testing; 2022. https://www.eucast.org/
38. CASFM: Comité de l'antibiogramme de la Société Française de Microbiologie; 2019. https://www.sfm-microbiologie.org/2019/07/09/casfm-veterinaire-2019
39. CLSI. *Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals*. 1st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
40. Dillane P, Krump L, Kennedy A, Sayers RG, Sayers GP. Establishing blood gas ranges in healthy bovine neonates differentiated by age, sex, and breed type. *J Dairy Sci*. 2018;101(4):3205-3212. doi:10.3168/jds.2017-13445
41. Song YY, Lu Y. Decision tree methods: applications for classification and prediction. *Shanghai Arch Psychiatry*. 2015;27:130-135. doi:10.11919/j.issn.1002-0829.215044
42. IBM Corp. *IBM SPSS Statistics for Windows: IBM SPSS Decision Trees*. Armonk, NY: IBM Corp; 2013.
43. Kushimoto S, Gando S, Saitoh D, et al. The impact of body temperature abnormalities on the disease severity and outcome in patients with severe sepsis: an analysis from a multicenter, prospective survey of severe sepsis. *Crit Care*. 2013;17(6):R271. doi:10.1186/cc13106
44. Ferreira FL, Bota DP, Bross A, Mélot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA*. 2001;286(14):1754-1758. doi:10.1001/jama.286.14.1754
45. Polat G, Ugan RA, Cadirci E. Sepsis and septic shock: current treatment strategies and new approaches. *Eurasian J Med*. 2017;49(1):53-58. doi:10.5152/eurasianjmed.2017.17062
46. Gustin P, Lomba F, Bakima J, Lekeux P, Van de Woestijne KP. Partitioning of pulmonary resistance in calves. *J Appl Physiol*. 1987;62(5):1826-1831. doi:10.1152/jappl.1987.62.5.1826
47. Pardon B, Hostens M, Duchateau L, Dewulf J, De Bleecker K, Deprez P. Impact of respiratory disease, diarrhea, otitis and arthritis on mortality and carcass traits in white veal calves. *BMC Vet Res*. 2013;9(1):79. doi:10.1186/1746-6148-9-79
48. Adams EA, Buczinski S. Short communication: Ultrasonographic assessment of lung consolidation postweaning and survival to the first lactation in dairy heifers. *J Dairy Sci*. 2016;99(2):1465-1470. doi:10.3168/jds.2015-10260
49. Wu J, Wang Y, Zhao A, Wang Z. Lung ultrasound for the diagnosis of neonatal respiratory distress syndrome: a meta-analysis. *Ultrasound Q*. 2020;36(2):102-110. doi:10.1097/RUQ.0000000000000490
50. Jourquin S, Bokma J, De Cremer L, et al. Randomized field trial comparing the efficacy of florfenicol and oxytetracycline in a natural outbreak of calf pneumonia using lung reaeration as a cure criterion. *J Vet Intern Med*. 2022;36(2):820-828. doi:10.1111/jvim.16348
51. Trefz FM, Feist M, Lorenz I. Hypoglycaemia in hospitalised neonatal calves: prevalence, associated conditions and impact on prognosis. *Vet J*. 2016;217:103-108. doi:10.1016/j.tvjl.2016.10.001
52. Boniatti MM, Cardoso PRC, Castilho RK, Vieira SRR. Is hyperchloremia associated with mortality in critically ill patients? A prospective cohort study. *J Crit Care*. 2011;26(2):175-179. doi:10.1016/j.jcrc.2010.04.013
53. Hussain SA, Uppal SK, Randhawa CS, Sood NK. Bovine intestinal obstruction: blood gas analysis, serum C-reactive protein and clinical, haematological and biochemical alterations. *J Appl Anim Res*. 2015;43(2):224-230. doi:10.1080/09712119.2014.963093
54. Nunes BP, Flores TR, Mielke GI, Thumé E, Facchini LA. Multimorbidity and mortality in older adults: a systematic review and meta-analysis. *Arch Gerontol Geriatr*. 2016;67:130-138. doi:10.1016/j.archger.2016.07.008
55. Gupta S, Sakhuja A, Kumar G, McGrath E, Nanchal RS, Kashani KB. Culture-negative severe sepsis: nationwide trends and outcomes. *Chest*. 2016;150(6):1251-1259. doi:10.1016/j.chest.2016.08.1460
56. Kethireddy S, Bilgili B, Sees A, et al. Culture-negative septic shock compared with culture-positive septic shock: a retrospective cohort study. *Crit Care Med*. 2018;46(4):506-512. doi:10.1097/ccm.0000000000002924
57. Liu A, Yo CH, Nie L, et al. Comparing mortality between positive and negative blood culture results: an inverse probability of treatment weighting analysis of a multicenter cohort. *BMC Infect Dis*. 2021;21(1):182. doi:10.1186/s12879-021-05862-w
58. Phua J, Ngerng W, See K, et al. Characteristics and outcomes of culture-negative versus culture-positive severe sepsis. *Crit Care*. 2013;17(5):R202. doi:10.1186/cc12896
59. Yang SC, Liao KM, Chen CW, Lin WC. Positive blood culture is not associated with increased mortality in patients with sepsis-induced acute respiratory distress syndrome. *Respirology*. 2013;18(8):1210-1216. doi:10.1111/resp.12121
60. Li Y, Guo J, Yang H, Li H, Shen Y, Zhang D. Comparison of culture-negative and culture-positive sepsis or septic shock: a systematic review and meta-analysis. *Crit Care*. 2021;25(1):167. doi:10.1186/s13054-021-03592-8
61. Paquette K, Sweet D, Stenstrom R, et al. Neither blood culture positivity nor time to positivity is associated with mortality among patients presenting with severe manifestations of sepsis: the FABLED cohort study. *Open Forum Infect Dis*. 2021;8(7). doi:10.1093/ofid/ofab321
62. Fecteau G, Smith BP, George LW. Septicemia and meningitis in the newborn calf. *Vet Clin North Am Food Anim Pract*. 2009;25(1):195-208, vii-viii. doi:10.1016/j.cvfa.2008.10.004
63. World Organisation for Animal Health (OIE). OIE list of antimicrobial agents of veterinary importance; 2019. https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/A_OIE_List_antimicrobials_July2019.pdf
64. Delaloye J, Calandra T. Invasive candidiasis as a cause of sepsis in the critically ill patient. *Virulence*. 2014;5(1):161-169. doi:10.4161/viru.26187
65. CLSI. *VET03. Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals*. 2nd ed. Wayne, PA: CLSI; 2020.
66. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis*. 2009;49(11):1749-1755. doi:10.1086/647952
67. Schumacher A, Vranken T, Malhotra A, Arts JJC, Habibovic P. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. *Eur J Clin Microbiol Infect Dis*. 2018;37(2):187-208. doi:10.1007/s10096-017-3089-2
68. Naseri A, Sen I, Turgut K, Guzelbektes H, Constable PD. Echocardiographic assessment of left ventricular systolic function in neonatal calves with naturally occurring sepsis or septic shock due to diarrhea. *Res Vet Sci*. 2019;126:103-112. doi:10.1016/j.rvsc.2019.08.009
69. Constable PD, Trefz FM, Sen I, et al. Intravenous and oral fluid therapy in neonatal calves with diarrhea or sepsis and in adult cattle. *Front Vet Sci*. 2021;7:603358. doi:10.3389/fvets.2020.603358
70. Lamy B, Dargère S, Arendrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the art. *Front Microbiol*. 2016;7:697. doi:10.3389/fmicb.2016.00697
71. European Committee on Antimicrobial Susceptibility Testing (EUCAST). *Intrinsic Resistance and Unusual Phenotypes*. EUCAST; 2007. https://www.eucast.org/
72. CLSI. Performance Standards for Antimicrobial Susceptibility Testing 2022; 32nd ed [Internet]; 2022. http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20M100%20ED32:2022&scope=user

73. The forty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO food additives series 39: Toxicological evaluation of certain veterinary drug residues in food. 1997 [Internet]; 1997.
74. CLSI. Performance Standards for Antimicrobial Susceptibility Testing 2021; 31st ed [Internet]; 2022.

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